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Edited by

K. VAS

Co-ordinating editor:

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# IN MEMORIAM K. VAS

The Editorial Board of *Acta Alimentaria* deeply regrets to announce that its founder and Editor-in-chief, Director of the Central Food Research Institute, President of theCommittee on Food Science of the Hungarian Academy of Sciences passed away on 22 November 1981 at the age of 62.

Professor VAS was an internationally recognized leading personality of Hungarian food science. His scientific heritage is of such a variety that it is impossible, within the frame of this obituary, to give a full account of it.

Professor VAS was born at Mosonmagyaróváron 20 August 1919. He graduated in 1941 as a chemical engineer at the Technical University in Budapest [1]. The research work he carried out as an assistant of the Food Chemistry Department of the Technical University (1941–1944) aroused interest [2, 3]. He received the degree of Doctor of Technical Sciences [4] in 1944. He continued his promising research activity, interrupted for some time by the Second World War, at the Research Institute for Agricultural Industry. While working at this Institute he was provided the possibility of several study tours abroad. In 1947–1948 he visited the University of Chicago, the University of California (USA) [5], Cambridge University and the Low Temperature Research Station, Cambridge (U. K.). The work carried out in the latter and his contact with Professor Maurice Ingram, developed during his residence there, had a decisive effect on his later career [6].

From the earliest days his principal interest was in food microbiology [13] and food preservation [2], [23]. His contributions to these fields became later the basis of scientific schools [23]. His further fields of activity included food chemistry, analytics, bio-engineering and applied enzymology [29, 36].

In 1948 he was appointed Head of the Department of Microbiology of the Research Institute of the Canning, Meat and Refrigeration Industries. He kept this position till 1959. Perhaps these years constituted the most active period of his research career. He also received his Candidate's degree (1952) and the degree of Academical Doctor of Sciences (1956) during this period [16].

His research in microbiology contributed greatly to the creation of the scientific foundations of food preservation, to the exploration of the mechanism of action of various physical and chemical antimicrobial factors [9, 10], of the damage to bacterial spores [15, 18, 19] and yeasts, and further of their kinetics of death [16, 17, 31]. He made his mark also in several other fields, e.g. in food analysis [8, 14, 20]; the engineering of enzyme preparations of microbial origin [11]; their application to food production; the development of certain analytical methods of food microbiology [7, 12]; and advocating the quantitative approach to microbiology in Hungary [37, 39–42]. The 18 innovations and patents of which he was author or co-author duely reflect his incentiveness and practical common sense.

In 1959 he was appointed Professor of Food Technology and Microbiology at the University of Horticulture. This appointment he held [25, 28]till 1967 when he became Director of the Central Food Research Institute.

In 1964 Prof. KÁROLY VAS was elected Corresponding Member of the Hungarian Academy of Sciences. The honorary degree of Doctor of the University of Horticulture was conferred on him in 1979.

His work at the University and later at the Institute was interrupted during the periods of 1964 to 1966 and 1972 to 1978 when as Head of the Food Preservation Section of the Joint FAO/IAEA Division of Atomic Energy in Food and Agriculture he organized international projects [27]. Food preservation by radiation treatment, of which he was one of the pioners in Eastern Europe [21, 22, 26, 30], remained his most important sphere of interest [35]. Ha was one of the greatest personalities in food preservation by radiation treatment. His project-organizing and diplomatic activities as researcher, international expert, consultant or adviser [32, 33] and particularly as United Nations officer, in the preparation of accepting and introducing on an international level the new process of food preservation by irradiation and in laying the foundations of the standardization of marketing irradiated commodities, are of inestimable value [34]. The fruits of these activities are maturing now inasmuch as this new technology gains ground in many countries.

KÁROLY VAS was a man in whom ability and knowledge was accompanied by an unceasing, ardent sense of responsibility, a sense of vocation and legendary industry. These qualities made him an outstanding scientist, an expert highly estimed the world over. However, he was more than an eminent researcher. The fact that he was a man of high integrity who had served the progress of his country and of all mankind to the utmost of his energies, made

him a man of stature. Thus, his pupils and colleagues may consider themselves lucky to have had him as their master and example not only as a scientist but also as a man.

He was a personality of exceptional leadership. His erudity and wide range of interests made him a true leader, a man of authority in a field as complex as food science. In him the researcher engrossed in the understanding and exploration of fundamental processes favourably alloyed the passion for the acquisition of knowledge and the ability of transmitting this knowledge and turning it to practical application with a deep sense of responsibility and with conscious assumption of his duty to society.

All these qualities made him the greatest organizer in Hungarian food science. To whichever field he devoted his energies he succeeded in creating lasting values. He professed and actively adopted the principle that science does not consist only of the acquisition of knowledge and observation of phenomena, but it also means that the results have to be made public property; this is proved by his 280 papers and 17 textbooks and lecture notes published. As founder, editor, or co-editor of various periodicals and journals he contributed to the propagation of scientific research at home and abroad and fought for the acceptance of Hungarian achievements. Hungarian research profited enormousi ly from his international connections.

He represented a similar driving force in many associations at home and abroad. He was member of the Executive Committee of the International Union of Food Science and Technology, the Management Committee of the International Union of Pure and Applied Chemistry, the presidium of the European Society of Nuclear Methods in Agriculture, the presidium of the Hungarian Scientific Society for the Food Industry, the Hungarian Microbiological Society, the Hungarian National Committee of PUGWASH, honorary member of the Union of Austrian Food and Biotechnologists. The acknowledgement of his scientific, organizing and associational activities is shown by the great number of awards and decorations he was honoured with.

As a man he was noble, ready to help, conscientious next to asceticism, and extremely modest. Always modest in questions touching his person while militant when representing matters of his field, the Institute, or the industry. During the whole of his career he aspired to produce work of quality. This claim for high quality was not mere word for him, it expressed his outlook on life as proved by his career, achievements, and the high level of his work. A remarkable sense of internal and external order and discipline was typical of KÁROLY VAS. In every position he held he was capable of inspiring his environment. Because of his manly reserve not everybody could come close to his personality, not even all his colleagues. The lucky ones who were on friendly terms with him could enjoy his erudition, his serenity and, always and in everything, his helpfulness.

His memory is cherished by his friends and many followers. His colleagues and pupils consider it their duty to follow in his steps and continue his work with the same intensity, devotion to work, and indefatigability as they had learned from him.

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# CORRELATION BETWEEN SENSORY AND GAS-CHROMATOGRAPHIC MEASUREMENTS ON ORANGE VOLATILES

J. PINO

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The relationship between gas-chromatographic data and sensory scores which were obtained by the hedonic scale method, was investigated in orange juice by simple linear regression analysis. According to the data given in this paper, there are some chromatographic peaks which appear to contribute substantially to the sensory feeling as indicated by the high value of the correlation coefficients between the sensory scores and the quantities of aroma compounds, such as myrcene, 2-hexanol, linalol and α-terpineol. Therefore, there exists the possibility of accurate estimation (with equations from multiple linear regression analysis) of particular aroma compounds to predict the sensory quality of orange juice.

The flavour of any processed food is a complex sensory property derived from the integrated effects of many compounds produced by chemical and enzymatic reactions. Adequate relationships have not yet been established between the sensory quality and the quantity of any compound on the gas chromatograms of most processed foods. Nevertheless, there has always been a great desire and a continuous effort to reduce subjective flavour responses to objective measurements and recently, numerous attempts have been made to evaluate the flavour quality of foods on the basis of whole gas chromatograms by applying multivariate analysis methods (PERSSON *et al.*, 1973; AISHIMA & NOBUHARA, 1977; SPENCER *et al.*, 1978).

The practical purpose for searching for such relations is the possibility of using instrumental techniques to complement or supplement sensory methods. Sensory methods often are tedious and sometimes suffer from subjectivity. The applications of these relations can be found in quality control work and in product and process development.

This paper attempts to find models for relationship between gas-chromatographic data and sensory scores which are exact enough for accurately predicting sensory responses from the gas-chromatographic data.

## 1. Materials and methods

### *1.1. Materials and processing*

Natural juice of sweet orange (*Citrus sinensis* L. Osbeck var. Valencia) was prepared in a *Lomi* manual juice extractor from four different lots of fruits (each lot represents 30 fruits).

Each lot of natural juice was divided into four samples and three of them were heated at different temperatures and for different periods (80 °C, 5 min; 90 °C, 2 min and 95 °C, 2 min) in closed glass bottles. The 16 samples were subjected to gas-chromatographic (GC) analysis and sensory test.

### *1.2. Aroma isolation and GC analysis*

The isolation and concentration of orange volatiles for GC analysis were performed by distillation of 500 $cm^3$ of juice in a vacuum rotary evaporator *(Rotadest)* and the distillate (400 $cm^3$) was trapped in one cold trap cooled with dry ice–acetone and 2 traps with liquid nitrogen. The volatile compounds were extracted from the distillate and the liquids in the traps with diethyl ether. Then, the extract was concentrated by careful distillation to 1 $cm^3$. The resulting volatile concentrate maintained the characteristics of the original aroma of each sample when it was diluted to the original volume.

The GC analysis was carried out on a *Packard–Becker* Model 419 equipped with a flame ionization detector. The separation was carried out on 0.4 cm o.d. $\times$ 3 m stainless steel columns packed with 60/80 mesh *Chromosorb* G coated with 5% polyethylene glycol adipate (BDH, England).

The GC conditions were: Argon carrier gas flow rate: 25 $cm^3 min^{-1}$; injector temperature: 220 °C; detector temperature: 250 °C. The best resolution was obtained with column temperature: 80 °C–200 °C at 5 °C $min^{-1}$. The peak areas were measured by a digital integrator *Autolab* 6300 and corrected by response factors (KEULEMANS, 1959).

### *1.3. Sensory evaluation*

The samples were evaluated using a hedonic scale method with 9-point preference scale for odour. In each test, 6 well-trained members of a sensory test panel evaluated the samples. The sum of scores produced for each sample was divided by the number of sensory test panel members and the quotient was used as the sensory score in this study. The same sensory test was repeated twice. The results were examined by analysis of variance and *Duncan* test (LERCH, 1967).

### *1.4. Mathematical methods*

The models for relating sensory scores ($S$) with gas-chromatographic data ($C$) are given in Table 1 where $C$ is a function of component concentrations ($X$) estimated by internal standard technique in GC analysis.

Table 1

*Models for relating sensory scores with gas-chromatographic data*

| | | |
|---|---|---|
| (I) | $S = a \cdot C + b$ | linear form |
| (II) | $\log S = a \cdot \log C + b$ | *Stevens'* law |
| (III) | $S = a \cdot \log C + b$ | *Fechner's* law |

| | | | |
|---|---|---|---|
| (1) | $C = X_i$ | (6) | $C = (X_i \cdot X_j)^{\frac{1}{2}}$ |
| (2) | $C = X_i - X_j$ | (7) | $C = (X_i + X_j)/X_k$ |
| (3) | $C = X_i + X_j$ | (8) | $C = X_i/X_j + X_k$ |
| (4) | $C = X_i + X_j + X_k$ | (9) | $C = X_i/X_j - X_k$ |
| (5) | $C = (X_i^2 + X_j^2)^{\frac{1}{2}}$ | (10) | $C = X_i/X_j$ |

$S$: sensory score
$C$: combination of peak concentrations
$X$: concentration data of peaks $i$, $j$, or $k$

The models used have been suggested by analogy with models used in other psychophysical contexts (PERSSON *et al.*, 1973). All the possible combinations of the peaks chosen were calculated. The validity of the different models was tested by means of simple linear regression analysis using a computer program with $F$ statistic *(Fischer)* as the criterion for adding or detaching a variable. The analysis was carried out by a *CID* 201 B computer (Cuba).

## 2. Results

A typical gas chromatogram of the orange volatiles isolated from juice is shown in Fig. 1. The identification of the major compounds was previously reported by PINO and co-workers (1980).

The 7 compounds used in the models are: α-pinene (peak 8), ethyl butyrate (peak 9), myrcene (peak 12), limonene (peak 14), 2-hexanol (peak 17), linalol (peak 22) and α-terpineol (peak 26). They were selected so that all of them changed in concentrations between different samples. Compounds, which do not change with heat treatment can be of importance only as "background" components, but are not important when analyzing how sensory quality is changed by heat treatment. In Table 2 are shown the concentration data of the selected orange volatile compounds used in the regression analysis.

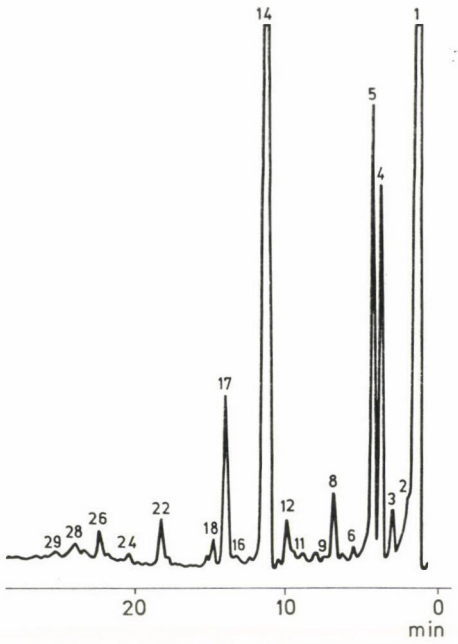


Fig. 1. Separation by gas chromatography of the orange volatile compounds isolated from juice on polyethylene glycol adipate column pack. Injector temperature: 220 °C; detector temperature: 250 °C; rate of the carrier gas: 25 $cm^3\ min^{-1}$. The temperature program was 80 °C to 200 °C at 5 °C $min^{-1}$

Using the models given in Table 1, simple linear regression analysis was carried out and the models with correlation coefficients higher than 0.80 are shown in Tables 3, 4 and 5.

Since the sensory tests are performed by scoring a positive correlation in a peak or peak combination means that the peak gives a preferable contribution with an increase in the quantity and a negative correlation means that the peak gives the reverse contribution. Of the 7 compounds used in the models, four gave high correlations. The concentration of 2-hexanol ($X_{17}$) shows a negative correlation with the sensory scores and this finding is very interesting because 2-hexanol is a newly found compound in orange juice (PINO *et al.*, 1980). The concentration of α-terpineol ($X_{26}$) also shows a negative correlation with the sensory scores in agreement with the reverse contribution of this compound in orange juice during storage (TATUM *et al.*, 1975). The concentration of linalol ($X_{22}$) is very important for the good quality of orange aroma due to the high correlations found in the three different models. Myrcene ($X_{12}$) also positively contributes to aroma quality.

Judging the resulting data, the linear model ($I$) generally describes the data best. It can also be seen that relations having peaks combined in vectorial

Table 2

*Concentration data ($\mu g/dm^3$) of the selected orange volatile compounds used in the regression analysis*

| Volatile compounds* | Heat treatment | | | |
|---|---|---|---|---|
| | not heated | | | |
| 8 | 1.9 ± 0.2 | 1.5 ± 0.1 | 1.5 ± 0.1 | 1.6 ± 0.1 |
| 9 | 0.3 ± 0.1 | 0.3 ± 0.1 | 0.2 ± 0 | 0.4 ± 0.1 |
| 12 | 4.4 ± 0.1 | 4.2 ± 0.1 | 3.9 ± 0.2 | 4.4 ± 0.2 |
| 14 | 655.0 ± 3.2 | 650.2 ± 2.2 | 643.4 ± 3.0 | 650.0 ± 2.3 |
| 17 | 2.8 ± 0.1 | 2.6 ± 0.1 | 2.6 ± 0.1 | 2.5 ± 0.1 |
| 22 | 14.4 ± 0.2 | 14.4 ± 0.1 | 13.8 ± 0.1 | 14.6 ± 0.2 |
| 26 | 3.3 ± 0.1 | 3.5 ± 0.1 | 3.5 ± 0.1 | 3.1 ± 0.1 |
| | heated at 80 °C for 5 min | | | |
| 8 | 1.7 ± 0.1 | 1.6 ± 0.1 | 1.6 ± 0.1 | 1.6 ± 0.1 |
| 9 | 0.3 ± 0.1 | 0.3 ± 0.1 | 0.2 ± 0 | 0.5 ± 0.1 |
| 12 | 4.3 ± 0.1 | 4.2 ± 0.1 | 3.8 ± 0.1 | 4.2 ± 0.1 |
| 14 | 650.0 ± 2.2 | 646.3 ± 2.0 | 639.1 ± 3.1 | 647.1 ± 3.2 |
| 17 | 2.9 ± 0.1 | 2.7 ± 0.1 | 2.7 ± 0.1 | 2.6 ± 0.1 |
| 22 | 13.6 ± 0.1 | 13.8 ± 0.2 | 13.9 ± 0.2 | 14.6 ± 0.2 |
| 26 | 3.3 ± 0.1 | 3.7 ± 0.1 | 3.6 ± 0.1 | 3.0 ± 0.1 |
| | heated at 90 °C for 2 min | | | |
| 8 | 1.4 ± 0.1 | 1.0 ± 0.1 | 1.2 ± 0.1 | 0.9 ± 0.1 |
| 9 | 0.2 ± 0 | 0.2 ± 0 | 0.1 ± 0 | 0.2 ± 0 |
| 12 | 3.8 ± 0.2 | 3.7 ± 0.1 | 3.5 ± 0.1 | 3.9 ± 0.1 |
| 14 | 645.0 ± 2.3 | 641.2 ± 2.4 | 635.2 ± 3.0 | 630.2 ± 3.1 |
| 17 | 3.1 ± 0.1 | 2.9 ± 0.1 | 3.0 ± 0.1 | 3.1 ± 0.1 |
| 22 | 13.0 ± 0.1 | 13.1 ± 0.1 | 13.4 ± 0.2 | 13.5 ± 0.1 |
| 26 | 5.3 ± 0.1 | 5.2 ± 0.1 | 5.3 ± 0.1 | 5.9 ± 0.1 |
| | heated at 95 °C for 2 min | | | |
| 8 | 1.0 ± 0.1 | 1.2 ± 0.1 | 1.2 ± 0.1 | 0.8 ± 0.1 |
| 9 | 0.2 ± 0 | 0.1 ± 0 | 0.1 ± 0 | 0.2 ± 0 |
| 12 | 3.7 ± 0.1 | 3.6 ± 0.2 | 3.5 ± 0.1 | 3.5 ± 0.1 |
| 14 | 640.1 ± 2.5 | 639.3 ± 2.1 | 635.2 ± 1.6 | 622.3 ± 3.5 |
| 17 | 3.2 ± 0.1 | 3.1 ± 0.1 | 3.0 ± 0.1 | 3.3 ± 0.1 |
| 22 | 12.9 ± 0.1 | 13.0 ± 0.1 | 13.4 ± 0.2 | 13.1 ± 0.1 |
| 26 | 7.8 ± 0.1 | 6.6 ± 0.1 | 5.3 ± 0.1 | 9.2 ± 0.1 |

* Compounds: 8: α-pinene; 9: ethyl butyrate; 12: myrcene; 14: limonene; 17: 2-hexanol; 22: linalool; 26: α-terpineol.

Table 3

*Linear regression models using the linear form (I)*

| $S = f(X)$ | $i$ | $j$ | $k$ | $r^*$ | $F^*$ |
|---|---|---|---|---|---|
| $X_i$ | 12 | — | — | 0.81 | 26.85 |
| | 17 | — | — | —0.83 | 31.44 |
| | 22 | — | — | 0.92 | 80.28 |
| $X_i - X_j$ | 22 | 17 | — | 0.80 | 22.74 |
| $(X_i^2 + X_j^2)^{\frac{1}{2}}$ | 8 | 12 | — | 0.84 | 33.57 |
| | 12 | 22 | — | 0.93 | 96.95 |
| $(X_i \cdot X_j)^{\frac{1}{2}}$ | 8 | 9 | — | 0.84 | 33.30 |
| | 12 | 14 | — | 0.80 | 22.84 |
| $X_i/X_j + X_k$ | 22 | 17 | 26 | —0.89 | 57.60 |
| | 14 | 17 | 26 | 0.86 | 40.64 |
| | 14 | 8 | 12 | —0.83 | 29.57 |
| $X_i/X_j - X_k$ | 22 | 26 | 9 | 0.89 | 53.22 |
| $X_i/X_j$ | 22 | 26 | — | 0.89 | 53.22 |
| | 14 | 17 | — | 0.83 | 32.11 |
| | 14 | 26 | — | 0.87 | 58.78 |

$S$: sensory score
$X$: concentration data of peak $i$, $j$ or $k$
* Reported values are significant at 1% probability level

summation (expression 5 in Table 1), geometric mean (expression 6 in Table 1) or in ratios (expressions 7–10 in Table 1) give higher correlation coefficients and $X$ values than arithmetic sums and differences (expressions 2–4 in Table 1). One possible explanation that the ratio combinations give good correlations may be that the components in the numerator are directly related to (positively correlated with) aroma quality and the components in the denominator are inversely related to (negatively correlated with) aroma quality. Thus, for example, in Tables 3 and 4, it can be seen that the combination $X_i/X_j$ is positively correlated with the sensory score when peak 22 is in the numerator [$r = 0.89$ in $S = f(X_{22})$] and peak 26 in the denominator [$r = -0.80$ in $S = f(X_{26})$].

The combinations from Table 1 with higher $F$ values were applied to multiple linear regression analysis and the models with higher $F$ values are shown in Table 6.

Table 4

*Linear regression models using* Stevens' *law (II)*

| $S = f(X)$ | $i$ | $j$ | $k$ | $r^*$ | $F^*$ |
|---|---|---|---|---|---|
| $X_i$ | 12 | — | — | 0.82 | 28.15 |
| | 17 | — | — | —0.80 | 23.90 |
| | 22 | — | — | 0.89 | 52.78 |
| | 26 | — | — | —0.80 | 21.68 |
| $X_i - X_j$ | 26 | 9 | — | —0.80 | 23.72 |
| $(X_i^2 + X_j^2)^{\frac{1}{2}}$ | 8 | 12 | — | 0.83 | 35.24 |
| | 12 | 22 | — | 0.91 | 59.58 |
| $(X_i + X_j)/X_k$ | 17 | 26 | 9 | —0.80 | 25.78 |
| $X_i/X_j + X_k$ | 22 | 17 | 26 | 0.82 | 28.78 |
| $X_i/X_j - X_k$ | 22 | 26 | 9 | 0.82 | 29.07 |
| $X_i/X_j$ | 22 | 26 | — | 0.80 | 25.00 |

*S:* sensory score
*X:* concentration data of peak *i*, *j* or *k*
* Reported values are significant at 1% probability level

Table 5

*Linear regression models using* Fechner's *law (III)*

| $S = f(X)$ | $i$ | $j$ | $k$ | $r^*$ | $F^*$ |
|---|---|---|---|---|---|
| $X_i$ | 12 | — | — | 0.81 | 26.31 |
| | 17 | — | — | —0.83 | 31.96 |
| | 22 | — | — | 0.93 | 87.76 |
| | 26 | — | — | —0.83 | 30.05 |
| $X_i - X_j$ | 26 | 9 | — | —0.84 | 32.95 |
| $(X_i^2 + X_j^2)^{\frac{1}{2}}$ | 8 | 12 | — | 0.84 | 33.74 |
| $X_i + X_j/X_k$ | 17 | 26 | 9 | —0.80 | 24.96 |
| $X_i/X_j + X_k$ | 22 | 17 | 26 | 0.87 | 42.82 |
| | 14 | 17 | 26 | 0.81 | 27.52 |
| | 14 | 8 | 12 | —0.83 | 32.16 |
| $X_i/X_j - X_k$ | 22 | 26 | 9 | 0.87 | 42.09 |
| $X_i/X_j$ | 22 | 26 | — | 0.85 | 36.32 |

*S:* sensory score
*X:* concentration data of peak *i*, *j* or *k*
* Reported values are significant at 1% probability level

Table 6

*Multiple linear regression models*

| Equations of the regression curves | $r^*$ | $F^*$ |
|---|---|---|
| **Equation 1** | | |
| $S = 37.97 + 1.99\,X_{12} + 1.42\,X_{22} - 0.09\,X_{14} - 0.28\,X_{26}$ | 0.98 | 55.61 |
| **Equation 2** | | |
| $S = 57.95 + 3.41\,\frac{X_{22}}{X_{17} + X_{26}} - 0.09\,X_{14}$ | 0.97 | 88.51 |

$S$: sensory score
$X$: concentration data of myrcene (peak 12), limonene (peak 14), 2-hexanol (peak 17), linalool (peak 22) or α-terpineol (peak 26)
* Reported values are significant at 1% probability level

The two models contain 4 independent variables and 3 of the variables occurred in both of the regression equations. These are: limonene ($X_{14}$), linalool ($X_{22}$) and α-terpineol ($X_{26}$). The first and the last compounds have negative coefficients or occurred in the denominator of a fraction, indicating a reverse contribution to aroma quality. The variable linalool ($X_{22}$) always has a positive coefficient indicating a positive contribution to aroma quality. In both regression equations the correlation coefficients are significant (1% level) and explain 96 and 94% of the observed aroma changes.

Table 7

*Comparison of the observed and calculated aroma scores*

| Equation | Observed score | Calculated score | Intercept | Slope | $r^*$ |
|---|---|---|---|---|---|
| 1 | 8.2 | 7.3 | 0.3 | 1.2 | 0.42 |
| | 4.8 | 4.5 | | | |
| | 7.8 | 6.7 | | | |
| | 5.0 | 4.2 | | | |
| | 7.8 | 6.7 | | | |
| | 5.4 | 4.9 | | | |
| 2 | 8.2 | 7.2 | 0.3 | 1.2 | 0.81* |
| | 4.8 | 5.0 | | | |
| | 7.8 | 7.3 | | | |
| | 5.0 | 4.9 | | | |
| | 7.8 | 7.9 | | | |
| | 5.4 | 5.4 | | | |

* Reported value is significant at 1% probability level

As a measure of how well the calculated scores agree with the observed scores, the correlation coefficient and the regression equation according to the two sets of data were calculated for each model. The results are shown in Table 7.

Ideally the slopes should be 1.0 and the intercepts 0, but the differences are so small that they are of no practical consequence. Thus, the calculated aroma scores using the gas chromatographic data are as accurate and representative as the aroma scores determined by the sensory test panel members.

The scope of this study precluded the repetition of the experiments with new samples and the search for curvilinear models. Work in these areas is in progress.

## 3. Conclusions

The results indicated that aroma quality in orange juice was linearly related to gas chromatographic data of some volatile compounds. Thus, linalool and myrcene make a positive contribution to orange aroma quality, but 2-hexanol and $\alpha$-terpineol have a negative contribution to orange aroma.

The high $F$ values and correlation coefficients obtained for several of the regression equations reported herein indicate that aroma quality of orange juice could be described and predicted from the relative concentrations of the volatile compounds measured by gas-chromatographic analysis.

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Address of the author:

Jorge PINO — Department of Fruit and Vegetables,
National Center for Scientific Research
Apartado 6880, Habana
Cuba

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# ATTEMPTS TO UTILIZE WHEY FOR THE PRODUCTION OF YEAST PROTEIN

## II. EFFECTS OF BIOTIN CONCENTRATION AND WHEY CONTENT AT CONSTANT LACTOSE CONCENTRATION

H. Nour El-Dien and A. Halász

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Whey was used in propagation of *Saccharomyces fragilis* in batch culture. The growth of *S. fragilis* was better in whey than in mineral medium. Addition of biotin to whey did not affect the specific growth rate, RNA content, protein content and yield. These characteristic values and lactose utilization decreased by decreasing the whey content at constant lactose concentration, while, addition of yeast extract of 0.5% to mineral medium produced higher values in specific growth rate, RNA content and protein content.

To meet, at least partially, the demands of the rapidly increasing population of the world, waste products of food and beverage industries should be upgraded. One of these industrial wastes is whey. Whey lactose can be converted into a more desirable nutrient like yeast protein. Technologies for yeast production on whey have been described in the literature (Amundson, 1967; Atkin *et al.*, 1967; Bechtle & Clayton, 1971; Castillo & Sánchez, 1978; Chapman, 1966; Porges *et al.*, 1951; Wasserman, 1960, 1961; Wasserman *et al.*, 1958, 1961).

The aim of this study was to investigate the effect of growth factors, such as biotin and yeast extract as a source of vitamins, on the propagation of *Saccharomyces fragilis* as carried out in batch culture.

### 1. Materials and methods

The microorganisms, culture media, cultivation methods, determination of the protein and nucleic acids content of yeast were the same as described previously (Nour El-Dien *et al.*, 1981).

## 2. Results and discussion

### *2.1. Effect of biotin*

This experiment was carried out in four column fermentors containing: (1) mineral medium + 2% lactose, (2) mineral medium + 2% lactose fortified with 150 $\mu$g $l^{-1}$ biotin, (3) whey medium (containing 2% lactose), supplemented with 0.5% from both ammonium sulphate and dibasic potassium phosphate, (4) whey medium (containing 2% lactose) supplemented with 0.5% from both ammonium sulphate and dibasic potassium phosphate and 150 $\mu$g $l^{-1}$ biotin, resp. As shown in Fig. 1, there was no significant difference between the growth in media (1) and (2) and in media (3) and (4), resp. (*i.e.* with or without biotin supplementation), but there was a highly significant difference between the growth of yeast in mineral media (1, 2) as compared to that in whey media (3, 4).

*2.1.1. The specific growth rate.* From results given in Table 1 it was clear that the specific growth rate ($k, h^{-1}$) of *S. fragilis* was higher (0.308) when whey medium was used (3) than in the case of a (1) mineral medium (0.156).

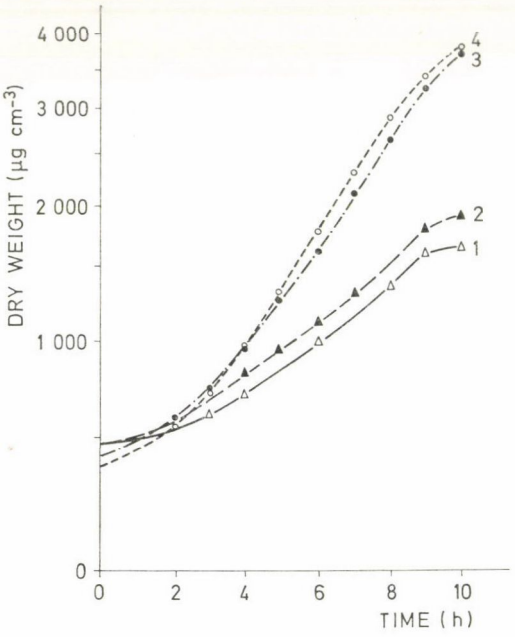


Fig. 1. The effect of medium composition on specific growth rate of *S. fragilis*
1 −△−△−△− mineral medium + 2% lactose;
2 −▲−▲−▲− mineral medium + 2% lactose + 150 $\mu$g $l^{-1}$ biotin;
3 −·−●−·−● whey 2% lactose + 0.5% $(NH_4)_2SO_4$ + 0.5% $K_2HPO_4$;
4 −○−○−○− whey 2% lactose + $(NH_4)_2SO_4$ + 0.5% $K_2HPO_4$ + 150 $\mu$g $l^{-1}$ biotin

Table 1

*The effect of medium composition on specific growth rate, yield, protein and RNA contents of* S. fragilis

| Media* | Specific growth rate ($k$) ($h^{-1}$) | RNA (% dry weight) | Protein (% dry weight) | Yield (%) |
|---|---|---|---|---|
| 1 | $0.156 \pm 0.025$ | $8.23 \pm 0.58$ | $41.60 \pm 0.76$ | $17.44 \pm 1.33$ |
| 2 | $0.160 \pm 0.025$ | $8.25 \pm 0.58$ | $41.65 \pm 0.76$ | $20.63 \pm 1.33$ |
| 3 | $0.303 \pm 0.025$ | $7.90 \pm 0.58$ | $42.20 \pm 0.76$ | $31.77 \pm 1.33$ |
| 4 | $0.308 \pm 0.025$ | $7.85 \pm 0.58$ | $42.40 \pm 0.76$ | $32.30 \pm 1.33$ |
| $F$ test for the columns | highly significant ($P \geq 99\%$) | not significant ($P < 95\%$) | not significant ($P < 95\%$) | highly significant ($P \geq 99\%$) |
| Least significant difference | 0.038 | — | — | 1.89 |

Average of 3 series
All values are $\bar{x} \pm t_{0.5} s_{\bar{x}}$

* Media:
(1) mineral medium + 2% lactose + 0.5% $(NH_4)_2SO_4$ + 0.5% $K_2HPO_4$
(2) mineral medium + 2% lactose + 0.5% $(NH_4)_2SO_4$ + 0.5% $K_2HPO_4$ + 150 $\mu g\ l^{-1}$ biotin
(3) whey (containing 2% lactose) + 0.5% $(NH_4)_2SO_4$ + 0.5% $K_2HPO_4$
(4) whey (containing 2% lactose) + 0.5% $(NH_4)_2SO_4$ + 0.5% $K_2HPO_4$ + 150 $\mu g\ l^{-1}$ biotin
RNA is the % of ribonucleic acid in the dry weight of harvested yeast
Protein is the % of protein in the dry weight of harvested yeast (% N×6.25)

$$\% \text{ yield} = \frac{\text{mg lactose utilized}}{\text{mg cell produced}} \times 100$$

On the other hand, there was no significant difference in specific growth rate in comparison to the original value when biotin (150 $\mu g\ l^{-1}$) was added to both media.

*2.1.2. The ribonucleic acid content of yeast.* The results (Table 1) indicated that the concentration of ribonucleic acid in the dry weight of harvested yeasts ranged from 7.8 to 8.3% and there was no significant difference between the ribonucleic acid content of yeast when grown on either medium (whey and mineral medium) even after addition of biotin.

*2.1.3. The protein content of yeast cell.* According to the results (Table 1) added biotin (150 $\mu g\ l^{-1}$) did not affect protein content of the dry harvested yeast in either medium.

*2.1.4. Cell yield on different media.* Yields are expressed as mg of cells produced per mg of lactose utilized, multiplied by 100. The results presented in Table 1 indicate low yields (17.44%) when *S. fragilis* was grown on mineral medium, but when this medium was supplemented with biotin the yield increased to 20.63%. There are significant differences between the yields in whey and in mineral medium, respectively. No significant differences in yield

were observed for biotin added to whey medium (150 $\mu g\ l^{-1}$), in contrast to the report by HARJU and co-workers (1976), who found that the supplementation of whey with biotin (30 mg $m^{-3}$) increased the yield from 22.0% to 31.0%. This result could be attributed to the biotin content of the whey powder (29.0 $\mu g$ biotin per 100 mg whey powder) used in the present experiment.

### *2.2. The effect of biotin concentration*

Previous data indicate no significant effect resulting from the addition of 150 $\mu g\ l^{-1}$ to the whey, in spite of several reports in the literature which showed that biotin served as a vital growth factor for yeast when it was fortified with it (HARJU *et al.*, 1976; DAVIES, 1964; SPICER, 1973). Therefore, this was investigated in further experiments (Table 2) which were carried out in 4 column fermentors by graded concentrations of biotin (0, 150, 450 and 750 $\mu g\ l^{-1}$, respectively).

Table 2

*The effect of different biotin concentrations on specific growth rate, yield, as well as protein and RNA contents of* S. fragilis

| Media* | Specific growth rate ($k$) ($h^{-1}$) | RNA (% dry weight) | Protein (% dry weight) | Yield (%) |
|---|---|---|---|---|
| 1 | $0.298 \pm 0.057$ | $7.9 \pm 0.086$ | $42.4 \pm 1.15$ | $32.03 \pm 0.43$ |
| 2 | $0.305 \pm 0.057$ | $8.2 \pm 0.086$ | $42.4 \pm 1.15$ | $33.43 \pm 0.43$ |
| 3 | $0.305 \pm 0.057$ | $8.1 \pm 0.086$ | $42.5 \pm 1.15$ | $33.78 \pm 0.43$ |
| 4 | $0.307 \pm 0.057$ | $8.1 \pm 0.086$ | $42.9 \pm 1.15$ | $32.97 \pm 0.43$ |
| $F$ test | N.S. | N.S. | N.S. | N.S. |

* Media:
(1) whey medium (containing 2% lactose) + 0.5% $K_2HPO_4$ + 0.5% $(NH_4)_2SO_4$
(2) whey medium (containing 2% lactose) + 0.5% $K_2HPO_4$ + 0.5% $(NH_4)_2SO_4$ + 150 $\mu g\ l^{-1}$ biotin
(3) whey medium (containing 2% lactose) + 0.5% $K_2HPO_4$ + 0.5% $(NH_4)_2SO_4$ + 450 $\mu g\ l^{-1}$ biotin
(4) whey medium (containing 2% lactose) + 0.5% $K_2HPO_4$ + 0.5% $(NH_4)_2SO_4$ + 750 $\mu g\ l^{-1}$ biotin
RNA is the % of ribonucleic acid in the dry weight of harvested yeast
Yield is expressed as mg of cells produced per mg of lactose utilized multiplied by 100
Protein is the % of protein in the dry weight of harvested yeast
All values are $\bar{x} \pm t_{0.5} s_{\bar{x}}$
N.S. = not significant ($P < 95\%$)

It is obvious from the results of Table 2 and Fig. 2 that no significant difference in specific growth rate, ribonucleic acid, protein content, and yield could be observed at different concentrations of biotin or without the addition of biotin. These results agree with our previous findings and are due to the high content of vital growth factor in whey.

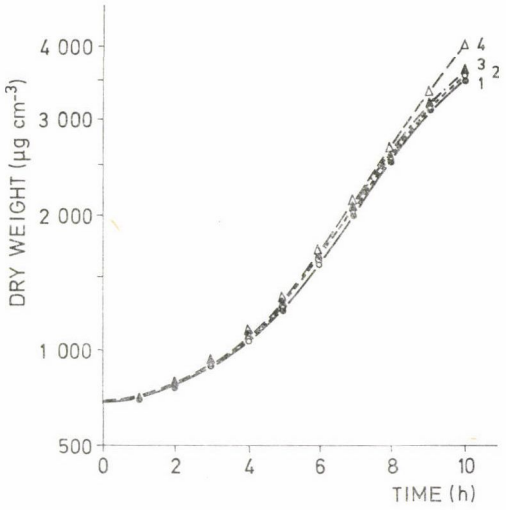


Fig. 2. Effect of biotin concentration on the growth rate of *S. fragilis*
1 —●—●—●— whey 2% lactose + 0.5% $K_2HPO_4$ + 0.5% $(NH_4)_2SO_4$;
2 —○---○---○— whey 2% lactose + 0.5% $K_2HPO_4$ + 0.5% $(NH_4)_2PO_4$ + 150 $\mu g\,l^{-1}$ biotin;
3 ---▲---▲---▲--- whey 2% lactose + 0.5% $K_2HPO_4$ + 0.5% $(NH_4)_2PO_4$ + 450 $\mu g\,l^{-1}$ biotin;
4 ---△---△---△--- whey 2% lactose + 0.5% $K_2HPO_4$ + 0.5% $(NH_4)_2SO_4$ + 750 $\mu g\ l^{-1}$ biotin

### *2.3. The effect of different contents of whey at constant lactose concentration and addition of yeast extract powder on the growth of* S. fragilis

This experiment was carried out in 6 column fermentors with media containing: (1) mineral medium + 2% lactose fortified with 150 $\mu g\ l^{-1}$ biotin, (2) mineral medium + 2% lactose fortified with biotin (150 $\mu g\ l^{-1}$) and yeast extract powder (0.5%), (3) whey medium containing 2% lactose, (4) whey medium containing 1% lactose + 1% pure lactose, (5) whey medium containing 0.5% lactose + 1.5% pure lactose, (6) whey medium containing 0.25% lactose + 1.75% pure lactose, each medium from 3 to 6 were also supplemented with nitrogen and phosphorus salts at the same concentration as in the mineral medium. The results were summarized in Table 4 and Figs. 3 and 4.

*2.3.1. Specific growth rate.* It can be seen from Table 3 and Fig. 3 that the specific growth rate of *S. fragilis* was decreased (0.303, 0.277, 0.230 and 0.195) by decreasing the whey content (original lactose content in whey medium 2%, 1%, 0.5% and 0.25% resp.) in spite of adding pure lactose to obtain 2% lactose in all media.

It can also be seen, from the results in Table 3 and Fig. 3, that the addition of yeast extract to mineral medium (0.5%) produced an about three-fold increase in specific growth rate (from 0.160 to 0.400). WASSERMAN and co-workers (1958) reported that addition of 0.1% yeast extract to whey produced a two-fold increase in specific growth rate.

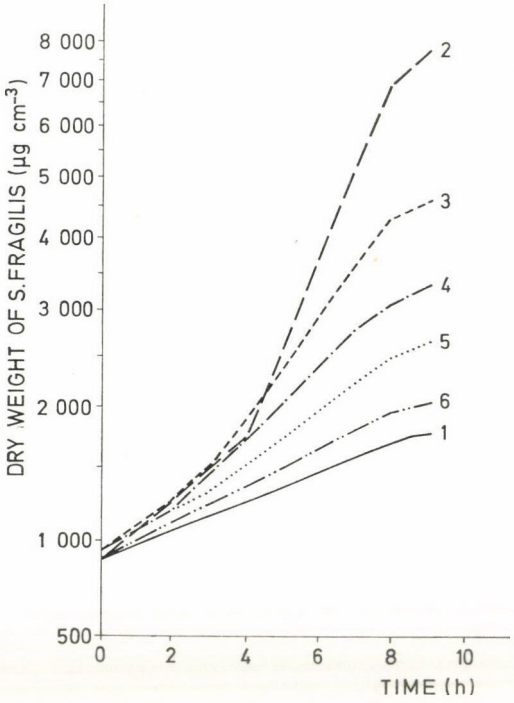


Fig. 3. Growth of *S. fragilis* in whey at different whey lactose concentrations. (Average of three series)

1 ——— mineral medium 2% lactose + biotin;
2 — — — mineral medium 2% lactose + biotin + yeast extract;
3 – – – – whey medium 2% lactose + N + P;
4 — · — · whey medium 1% lactose + N + P;
5 ------ whey medium 0.5% lactose + 1.5% pure lactose + N + P;
6 —··—·· whey medium 0.25% lactose + 1.75% pure lactose + N + P

*2.3.2. The ribonucleic acid content in* S. fragilis. The results in Table 3 indicate a relation between the specific growth rate and the ribonucleic acid content of yeast. The increase in specific growth rate was accompanied by an increased ribonucleic acid content of yeast. However, in case of mineral medium (1) a higher RNA content of yeast correlates with a lower specific growth rate as in case of whey (6).

*2.3.3. The protein content of yeast.* The results which are presented in Table 3 show a very slight decrease in the protein content (dry weight basis) of harvested yeast parallel to the decrease in the original whey lactose content (whey content) in the medium. But an addition of yeast extract to the mineral medium caused a considerable increase in yeast protein content to 50.68%.

*2.3.4. Lactose utilization and yield coefficient.* As it can be seen in Fig. 4 and Table 3, more than 87% of lactose was utilized after 8 h in the mineral medium containing 0.5% yeast extract (this is in agreement with the findings of

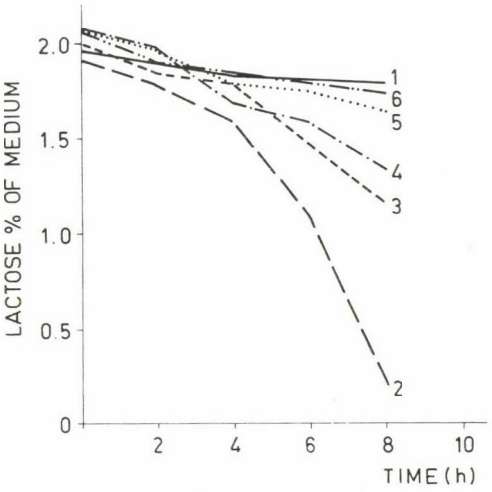


Fig. 4. Lactose content during fermentation period in different media
1 ——— mineral medium 2% lactose + 150 $\mu$g $l^{-1}$ biotin;
2 — — mineral medium + 150 $\mu$g $l^{-1}$ biotin + 0.5% yeast extract;
3 — — — whey medium 2% lactose + N + P;
4 — · — · whey medium 1% lactose + 1.0% pure lactose + N + P;
5 - - - - - - whey medium 0.5% lactose + 1.5% pure lactose + N + P;
6 —··—·· whey medium 0.25% lactose + 1.75% pure lactose + N + P (Average of three series)

Table 3

*The effect of medium composition on specific growth rate, protein and RNA contents of* S. fragilis *and on cell yield*

| Media* | Specific growth rate ($k$) ($h^{-1}$) | RNA (%) | Protein (%) | Lactose utilization | Yield (%) |
|---|---|---|---|---|---|
| 1 | 0.160 ± 0.026 | 7.6 ± 0.126 | 41.60 ± 0.75 | 8.50 ± 0.18 | 21.12 ± 0.57 |
| 2 | 0.400 ± 0.026 | 10.6 ± 0.126 | 50.68 ± 0.75 | 87.18 ± 0.18 | 39.82 ± 0.57 |
| 3 | 0.303 ± 0.026 | 7.5 ± 0.126 | 41.72 ± 0.75 | 41.46 ± 0.18 | 41.53 ± 0.57 |
| 4 | 0.277 ± 0.026 | 7.0 ± 0.126 | 41.53 ± 0.75 | 29.76 ± 0.18 | 38.88 ± 0.57 |
| 5 | 0.230 ± 0.026 | 6.3 ± 0.126 | 41.43 ± 0.75 | 20.00 ± 0.18 | 38.03 ± 0.57 |
| 6 | 0.195 ± 0.026 | 5.3 ± 0.126 | 40.77 ± 0.75 | 15.24 ± 0.18 | 35.00 ± 0.57 |
| *F* test | highly significant (P ≥ 99%) | highly significant (P ≥ 99%) | highly significant (P ≥ 99%) | highly significant (P ≥ 99%) | highly significant (P ≥ 99%) |
| Least significant difference | 0.036 | 0.178 | 1.07 | 0.25 | 0.82 |

* Media:
(1) mineral medium + 2% lactose + 150 $\mu$g $l^{-1}$ biotin
(2) mineral medium + 2% lactose + 150 $\mu$g $l^{-1}$ biotin + 0.5% yeast extract powder
(3) whey medium containing 2% lactose + N & P at the same concentration as in mineral medium
(4) whey medium containing 1% lactose + 1% pure lactose + N & P at the same concentration as in mineral medium
(5) whey medium containing 0.5% lactose + 1.5% pure lactose + N & P at the same concentration as in mineral medium
(6) whey medium containing 0.25% lactose + 1.75 pure lactose + N & P at the same concentration as in mineral medium

N = $(NH_4)_2SO_4$ 0.4%
P = $KH_2PO_4$ 0.014%
$Na_2HPO_4 \cdot 12\ H_2O$ 0.08%

Yield and lactose utilization are calculated after 8 h of fermentation

CASTILLO & SÁNCHEZ, 1978). We also observed that by lowering the original lactose content in whey (whey content), the lactose utilization was decreased. Lactose utilization shows a correlation with the yield coefficient: decreasing the original whey lactose content (whey content) caused a decrease in the yield.

## 3. Conclusions

*Saccharomyces fragilis* was propagated in whey. These studies were carried out in column fermentors in a batch culture.

It was noticed that the specific growth rate ($k, h^{-1}$) and yield of *S. fragilis* was higher in whey than in the mineral medium, but there was no significant difference between the protein and ribonucleic acid content (on dry weight) of harvested yeast, in the two media.

Addition of biotin to whey did not affect the specific growth rate, ribonucleic acid content, protein content and yield.

On the other hand, these characteristic values and lactose utilization decreased by decreasing the original whey lactose content (whey content).

Although it was obvious that addition of yeast extract of 0.5% to mineral medium produced higher values in specific growth rate, ribonucleic acid content, protein content and yield.

Thus, it is suggested that whey contains some vital factors other than biotin which improve the growth of *S. fragilis*, and these vital factors also seem to be present in yeast extract.

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Address of the authors:

Dr. Anna HALÁSZ
Hassan NOUR EL-DIEN*

Central Food Research Institute
H-1022 Budapest, Herman Ottó út 15.
Hungary

* Present address:

Dairy Department,
Faculty of Agriculture,
Tanta University
Kafr el-Sheikh, Alexandria. Egypt

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# FACTORS AFFECTING PECTIN LYASE ACTIVITY

K. ZETELAKI-HORVÁTH

(Received: 24 June 1980; accepted: 21 August 1980)

The pectin lyase component (PL) of a pectolytic enzyme preparation obtained by fermentation with *Aspergillus niger* was studied.

The optimum incubation temperature was found to be 40 °C while the optimal pH was 6.0.

In the concentration range of 0.01–0.1 mg $cm^{-3}$ enzyme preparation; the reaction rate increased linearly if an incubation period of 30 min or less was applied. On increasing the substrate concentration within the range 1–10 mg $cm^{-3}$, the reaction rate increased again linearly.

From the data of reaction rate *vs* substrate concentration the kinetic constants were calculated and $V_{max}$ was found to be 0.169 $min^{-1}$, while $K_m$ was 32.96 mg $cm^{-3}$.

In the course of degradation of the pectin molecule by transelimination without water uptake, the final product of lyase-type pectolytic enzymes is unsaturated galacturonic acid or its oligomers (Fig. 1).

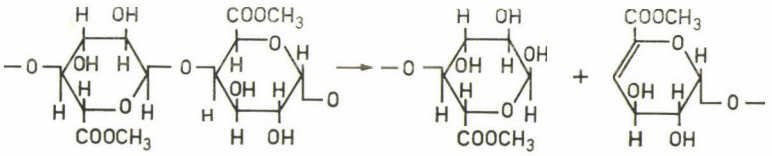


Fig. 1. Mechanism of pectin lyase action

The pectin lyase enzymes (PL) attack the pectin chain of high methyl-ester content either at the end of the chain (exo-PL) or at random (endo-PL). For the breakdown of slightly esterified pectins or of pectic acid, the exo- or endopectic acid lyases (PAL) are responsible.

The pectin lyase enzyme is generally produced by various fungal strains, mainly belonging to the *Aspergillus* species (*A. fonsecaeus*: EDSTROM & PHAFF, 1964; *A. niger*: VASU, 1967; *A. japonicus*, *A. soyae*: ISHII & YOKOTSUKA, 1975; ISHII, 1976; or *Sclerotinia fructigena*: BYRDE & FIELDING, 1968), while the pectate or pectic acid lyases are synthesized by bacteria (ZUCKER & HANKIN, 1970; DAVÉ *et al.*, 1976; KAMIMIYA *et al.*, 1977).

There is a substantial difference in the pH-optima of pectin lyase and pectate lyase, the first being at 5.5–6.0, while the second at 8.5–9.0.

Pectin lyase was isolated from several commercial pectolytic enzyme products (AMADO, 1970; VORAGEN, 1972).

Pectin lyase enzymes obtained from *Aspergillus japonicus* and *Aspergillus soyae* have a very high clarifying effect, but exert also a macerating action (ISHII & YOKOTSUKA, 1971a, b, 1973; ISHII, 1976).

## 1. Materials and methods

### *1.1. Conditions of culturing*

The microorganism used was an *Aspergillus niger* variant isolated after treatment with Na-azide (ZETELAKI-HORVÁTH *et al.*, 1979).

Fermentation was carried out in 10-l glass fermentors (KUTESZ, Hungary) at 32 °C. The carbon source in the medium was a natural pectin inducer (beet extract). Six liter of medium were inoculated with 600 $cm^3$ of a 24-h vegetative culture.

### *1.2. Extraction of the enzyme*

The enzyme preparation was obtained by precipitation with alcohol from the 72 h old fermentation broth. The specific activity was: 120 $\mu$mol $min^{-1}$ $g^{-1}$.

### *1.3. Kinetic test of the enzyme*

The aim of the kinetic analysis was to establish the optimum parameters of PL activity and the kinetic constants.

### *1.4. Measurement of enzyme activity*

The substrate used for enzyme activity measurement was *Pomosin* pectin [(Degree of esterification = E = 70%) (POMOSIN WERKE, GmbH, FRG)]. Enzyme activity was determined spectrophotometrically, based on the light absorption of double bonds formed as an effect of PL and having an absorption maximum at 235 nm. The activity was calculated from the difference in absorption of the enzyme-containing and the control sample, applying the molar extinction coefficient value of 5 500:

$$PL = \frac{(OD_M - OD_K) \times 10\,000 \times H}{5\,500 \times 15} = 0.1212\ (OD_M - OD_K) \times H\ \mu mol\ min^{-1}$$

where:

$OD_M$ = optical density of the enzyme containing sample,
$OD_K$ = optical density of the control sample,
5 500 = molar extinction coefficient of the unsaturated methylated galacturonic acid,
15 = reaction period (min),
$H$ = dilution factor.

PL activity was characterized by the amount in $\mu$mol of unsaturated galacturonic acid formed per min.

### *1.5. Mathematical statistical evaluation*

Measurements were carried out in 3–4 repetitions. The deviation around the mean was calculated. The kinetic constants ($V_{max}$ and $K_m$) were determined according to LINEWEAVER and BURK or regression calculations.

## 2. Results

### *2.1. Effect of temperature*

The effect of temperature was studied in the temperature range of 20–50 °C in media of different enzyme concentrations (0.1, 0.5 and 1.0 mg $cm^{-3}$).

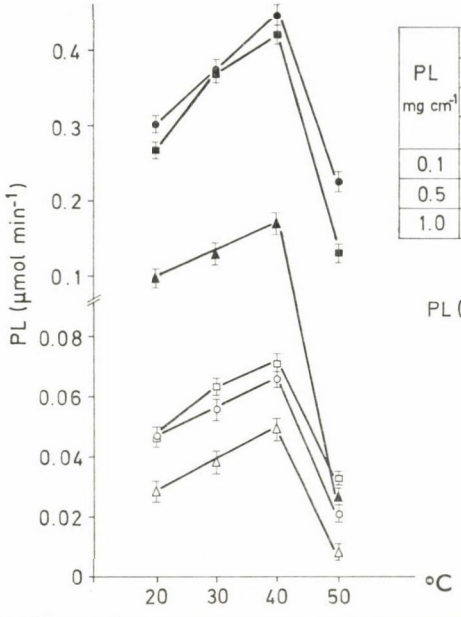
At the beginning of the work 120 min incubation periods were used according to ALBERSHEIM and co-workers (1960) but measurements were changed over to and repeated with reaction periods of 15 min. The substrate concentration was 10 mg $cm^{-3}$ and the pH 6.0 (Fig. 2).

At all three enzyme concentrations and both incubation periods, 40 °C proved to be the optimal temperature. The fact that at the enzyme concentration of 0.5 mg $cm^{-3}$ the activity was higher than at 1 mg $cm^{-3}$ is probably due to the inhibitory effect of the higher amounts of reaction products at the higher enzyme concentration.

### *2.2. Effect of pH*

The effect of pH was investigated in the pH range of 3.5–7.0. The substrate concentration was 10 mg $cm^{-3}$ and the enzyme concentration 1 mg $cm^{-3}$. Measurements were carried out at two different temperatures (at 30 and 40 °C) and three incubation periods (15, 60 and 120 min) (Fig. 3).

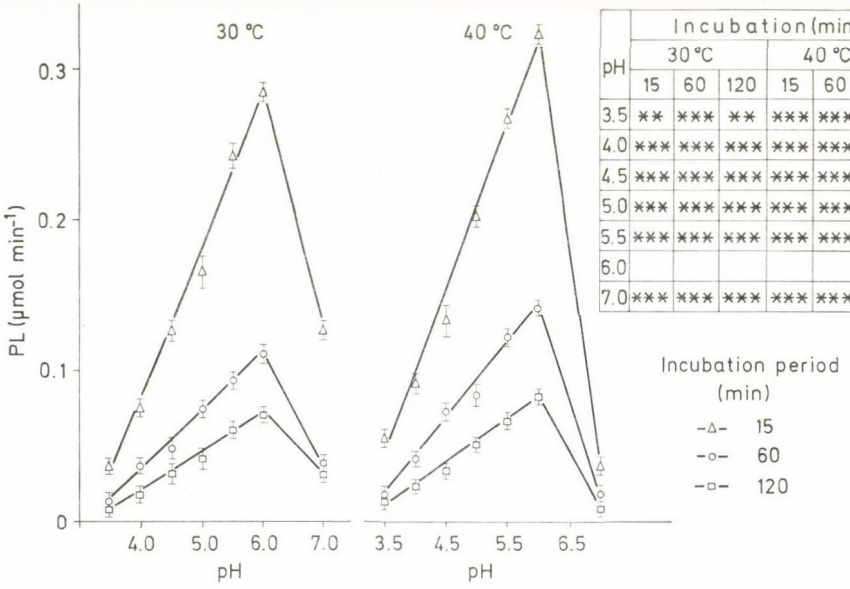
The PL activity proved to be very highly significantly higher at pH 6.0 than at any other pH at both temperatures and all three incubation periods.




| PL mg cm⁻³ | Incubation period (min) 15 °C | | | | 120 °C | | | |
|---|---|---|---|---|---|---|---|---|
| | 20 | 30 | 40 | 50 | 20 | 30 | 40 | 50 |
| 0.1 | *** | * | | *** | *** | * | | *** |
| 0.5 | *** | ** | | *** | *** | * | | *** |
| 1.0 | *** | *** | | *** | *** | ** | | *** |

| PL (mg cm⁻³) | Incubation (min) 15 | 120 |
|---|---|---|
| 0.1 | △ | ▲ |
| 0.5 | □ | ■ |
| 1.0 | ● | ● |

Fig. 2. Effect of temperature on pectin lyase activity. Comparison by $t$ test of activities as measured at different temperatures and the optimum temperature
* = significant ($P \geq 95\%$)
** = highly significant ($P \geq 99\%$)
*** = very highly significant ($P \geq 99.9\%$)




| pH | Incubation (min) 30 °C 15 | 60 | 120 | 40 °C 15 | 60 | 120 |
|---|---|---|---|---|---|---|
| 3.5 | ** | *** | ** | *** | *** | *** |
| 4.0 | *** | *** | *** | *** | *** | *** |
| 4.5 | *** | *** | *** | *** | *** | *** |
| 5.0 | *** | *** | *** | *** | *** | *** |
| 5.5 | *** | *** | *** | *** | *** | *** |
| 6.0 | | | | | | |
| 7.0 | *** | *** | *** | *** | *** | *** |

Fig. 3. Effect of pH on PL activity. Comparison by $t$ test of activities as measured at different pH values and the optimum value
** = highly significant ($P \geq 99\%$)
*** = very highly significant ($P \geq 99.9\%$)

*2.3. Effect of enzyme concentration*

The effect of enzyme concentration was investigated in the range of 0.01–2 mg $cm^{-3}$ during six different incubation periods (15–120 min). Measurements were carried out at pH 6.0 and 40 °C (Fig. 4).

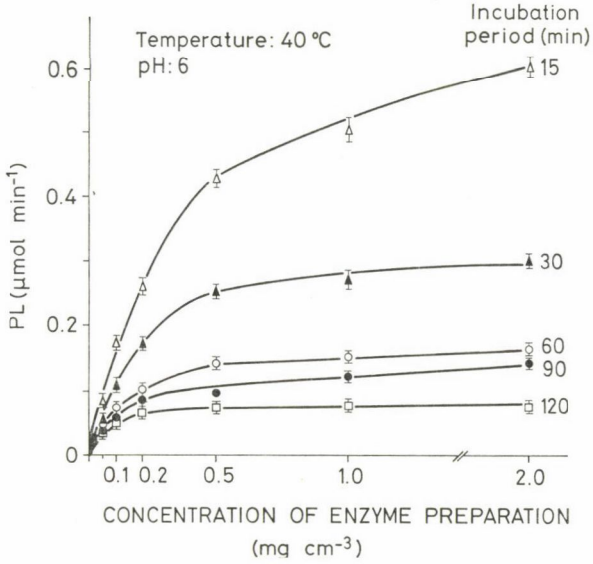



Fig. 4. Effect of enzyme concentration and incubation period on PL activity. Substrate concentration: 10 mg $cm^{-3}$

In the range of 0.01–0.1 mg $cm^{-3}$ enzyme preparation concentration, PL activity increased linearly with increasing concentration when incubation periods of 15 or 30 min were applied. During longer incubation periods, the linear section ended at 0.1 mg $cm^{-3}$ enzyme concentration.

*2.4. Effect of substrate concentration*

The effect of substrate concentration was studied in the range of 1–10 mg $cm^{-3}$ applying six different incubation periods (15, 30, 45, 60, 75 and 90 min) at a pH of 6.0 and 40 °C. The enzyme concentration was 1.0 mg $cm^{-3}$ (Fig. 5).

During incubation periods of 15 min, increasing the substrate concentration from 1.0 to 5 mg $cm^{-3}$ increased the enzyme activity about three-fold. With further increase of the substrate concentration the increase in the enzyme activity diminished. During incubation periods of 30 and 45 min the increase of substrate concentration up to 10 mg $cm^{-3}$ caused a linear increase in enzyme activity, however, this was substantially lower than during a 15 min

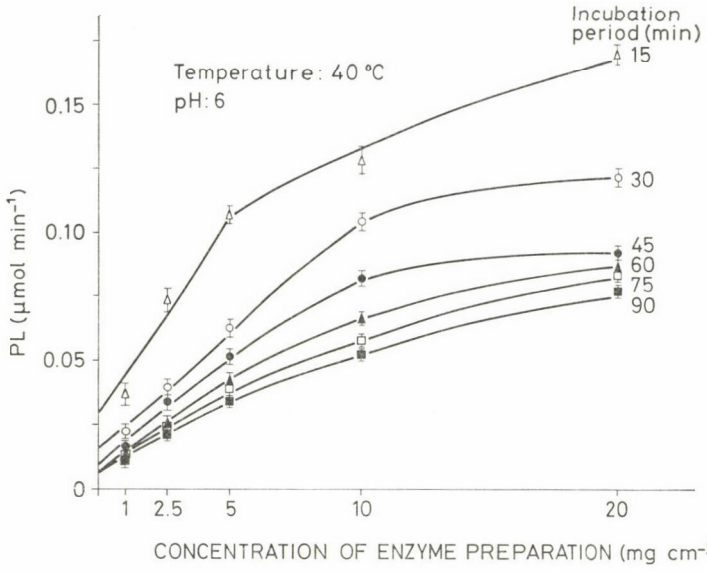



**Fig. 5. Effect of substrate concentration and incubation period on PL activity. Enzyme concentration: 1 mg cm$^{-3}$**

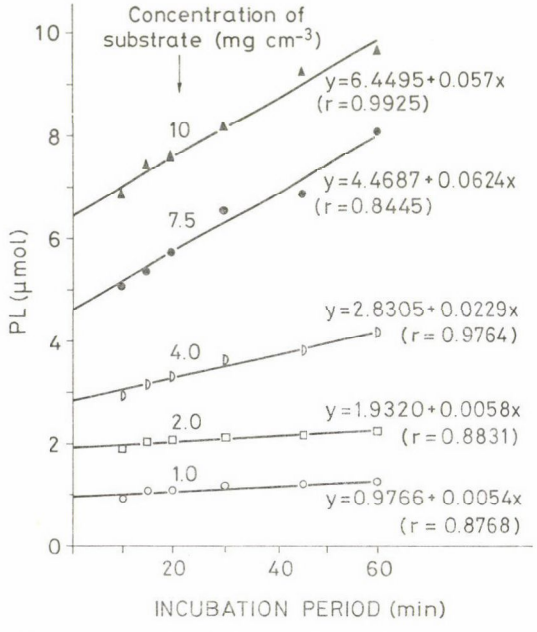



**Fig. 6. Effect of incubation period on PL activity at different substrate concentrations**

incubation period. During incubation periods of 60 and 90 min the increase in enzyme activity as a function of substrate concentration was significantly lower.

PL activity as a function of substrate concentration in the range of 1–10 mg $cm^{-3}$ was determined by regression analysis (Fig. 6).

When the substrate concentration was changed from 1 to 2.0 mg $cm^{-3}$, only a slight change of reaction rate was observed. By increasing the concentration from 2.0 to 4.0 or 10 mg $cm^{-3}$ the reaction rate increased nearly four-fold or ten-fold, resp.

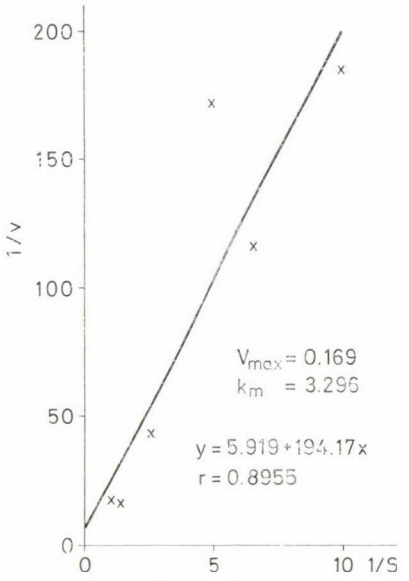



Fig. 7. Graphical representation according to *Linewaver-Burk* of PL activity *vs.* substrate concentration

### *2.5. Determination of the enzyme kinetic constants*

By graphically representing the reciprocal values of reaction rates as a function of the reciprocal of substrate concentration (Fig. 7), the *Michaelis* constant ($K_m$) and the maximal reaction rates were determined.

The maximum of the reaction rate was found to be ($V_{max}$) 0.1689 $\mu$mol $min^{-1}$, while the *Michaelis* constant was 3.296 mg $cm^{-3}$.

## 3. Conclusions

As a result of the reaction kinetic measurements, instead of the 120 min incubation period as suggested by ALBERSHEIM and co-workers (1960), the initial reaction rate was measured during 15 min.

In the course of the present study, the pH optimum of PL, obtained from *Aspergillus niger* at our Institute, was found to be 6.0. This value corresponded to that of the enzyme obtained by ISHII and YOKOTSUKA (1975) from *Aspergillus japonicus*. The optimum value of the PL purified by VORAGEN (1972) from commercially available preparations was at pH 6.1–6.5.

The optimum temperature of the enzyme was observed at 40 °C. At 50 °C the enzyme activity decreased substantially.

It was found that, for enzyme activity measurements, the use of the PL preparation in a concentration of 0.05–0.2 mg per $cm^3$ of reaction mixture is advisable.

The reaction constants as determined after the reaction kinetic measurements did not differ substantially from the values found in the literature. AMADO (1970) measured a $K_m$ value of 2.17 mg $cm^{-3}$ in the case of a purified PL preparation, while that of a technical PL preparation, produced at the CENTRAL FOOD RESEARCH INSTITUTE from *Aspergillus niger* culture, was found to be 3.296 mg $cm^{-3}$.

VORAGEN (1972) found that the value of $V_{max}$ of PL was independent of the degree of esterification of the substrate. He found further that the affinity of the enzyme to the substrate, which is proportional to $K_m^{-1}$ is highly dependent on temperature.

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Address of the author:

Dr. Kornélia ZETELAKI-HORVÁTH Central Food Research Institute
H-1022 Budapest, Herman Ottó út 15.
Hungary

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# EFFECT OF A SYNTHETIC DIHYDROCHALCONE SWEETENER (CHINOIN-401) ON CARBOHYDRATE METABOLISM

P. Szabó, T. Ferenczy, É. Bolla-Pusztai and J. Szejtli

(Received: 12 September 1980; accepted: 6 January 1981)

The synthetic sweetener CH-401 was examined for influence on some parameters of carbohydrate metabolism by oral administration to rabbits at the dose levels of 50 and 250 mg per kg bwt, for 30 days. The experimental observations permitted the following conclusions:

— Treatment was followed by an insignificant change in the blood glucose level. The fluctuations were within the physiological range.

— The hepatic concentration of trichloroacetyl acid soluble glycogen was not changed significantly by the treatment. Rabbits treated at the higher dose level showed a definite decrease in total hepatic glycogen concentration.

— CH-401 caused an increase in the hepatic G-6-P-ase enzyme activity at both levels of treatment.

— The pancreatic *Langerhans* islets of the treated animals showed no microscopic lesion.

— In 24 h urine glycosuria could not be proved by the *Fehling* reaction.

— Our experimental data seem to show that, under the given experimental conditions, neither an oral treatment of longer duration, nor a single intraperitoneal treatment induce alterations indicative of diabetes.

During the synthesis of dihydrochalcone series, Farkas and co-workers (1973) noticed that one compound had an intensive sweet taste. This compound [1-/2-hydroxy-4-(3-sulpho-propyloxy)phenyl /-3-/3-hydroxy-4-methoxy phenyl/-propanon-1-Na] was designated as *CHINOIN*-401. It proved to be non-toxic in oral toxicity tests performed in rats.

Rajky-Medveczky and co-workers (1977) and Lindner and co-workers (1977) observed that in aqueous solution, 200 to 1 500 times lower quantities of the compound produced the same sweet taste as the saccharose solution used for reference. The intensity of the taste was concentration-dependent. Testing of the compound in various foods showed that it could be used for the sweetening of products in which the sweet after-taste does not interfere with palatability (tinned jams for diabetics, non-alcoholic beverages, *etc.*) and for the sweetening of animal feeds as well.

This prompted closer investigations into the influence of *CHINOIN*-401 (CH-401) on carbohydrate metabolism.

## 1. Materials and methods

The experiments were performed in rabbits *(Oryctolagus cuniculus)* weighing 1 800–2 500 g. The control animals were fed a pelleted rabbit diet (LATI, Gödöllő) at a standard individual daily ration of 150 g. The experimental food was prepared as follows: after having been pulverized finely by grinding the standard food preparation was blended with CH-401 of 50 mg per kg, or 250 mg per kg, resp., according to the daily need and the body weight of the animals. To avoid loss of material, the mixture was regranulated. The feeding experiment lasted 30 days.

The blood glucose level was determined on 10 occasions during the period of the experiment. The fasting blood glucose level was measured with HULTMAN's (1959) *o*-toluidine reaction spectrophotometrically. In the urine collected during 24 h *Fehling*'s reduction test was performed daily. At the end of the experiment, the hepatic TCA-soluble glycogen fraction (extractable with 10% trichloroacetic acid) was determined. The KOH-soluble, so-called total glycogen was extracted by the method of KITS VON HEININGEN and KEMP (1954). Quantitative determination was carried out with the spectrophotometric procedure proposed by ROE (1955).

The glucose-6-phosphatase (G-6-P-ase) activity of the liver was determined by measuring the inorganic phosphate released from glucose-6-P (FARKAS *et al.*, 1973; LINDNER *et al.*, 1977).

After conclusion of the treatment, the rabbits were killed by bleeding, and the pancreas of each animal was removed for histological examination. For details of histological processing see the legend to the Figure 5.

In another series of experiments, 50 and 250 mg of CH-401 were injected per kg bwt, intraperitoneally in rabbits starved during 24 h. The blood glucose concentration was determined at hourly intervals, in the urine *Fehling* test was performed.

Mathematical-statistical evaluation of the experimental results was performed with *Student*'s test (WEBER, 1964).

## 2. Results

The blood glucose levels determined at different times during treatment with CH-401 are shown in Fig. 1. The values were related to the concentration measured before treatment as the base-line. In the initial stage of CH-401 treatment the blood glucose level showed an insignificant elevation. The slight increase in blood glucose concentration may be interpreted as stress effect caused by the operative intervention. On the 7th day and on the 11th day the blood glucose level was normal. It was concluded that the prolonged

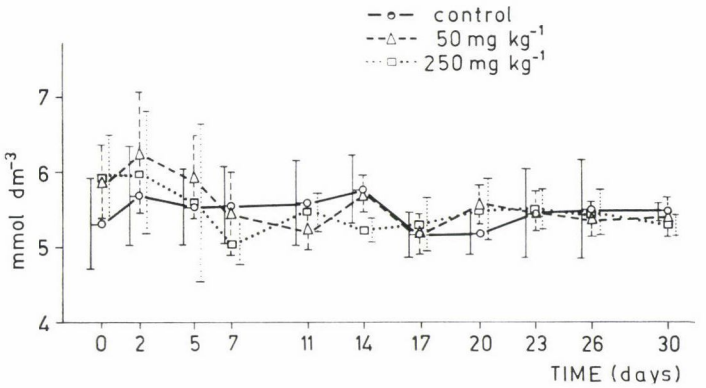


Fig. 1. Changes in the blood glucose level with standard deviations in rabbits treated orally with CH-401 for 30 days

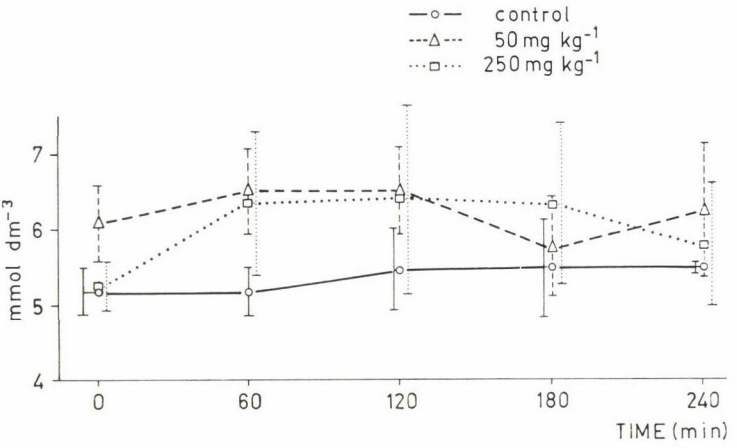


Fig. 2. Data of blood glucose concentration of rabbits treated intraperitoneally

administration of CH-401 at high dose levels did not give rise to a diabetic degree of hyperglycaemia. The results obtained by the intraperitoneal treatments are similar to the above-mentioned ones, too. During the observation period the blood glucose level remains normal (Fig. 2). In 24 h urine, glycosuria could not be proved by the *Fehling* reaction.

Figure 3 shows the influence of CH-401 treatment on the concentration of the hepatic glycogen fractions. The easily mobilizable TCA-soluble glycogen fraction showed no concentration change during the period of observation. It could be calculated that the so-called bound hepatic glycogen fraction (= difference between total and TCA-soluble) was significantly decreased after 30 days of treatment at 250 mg per kg bwt level.

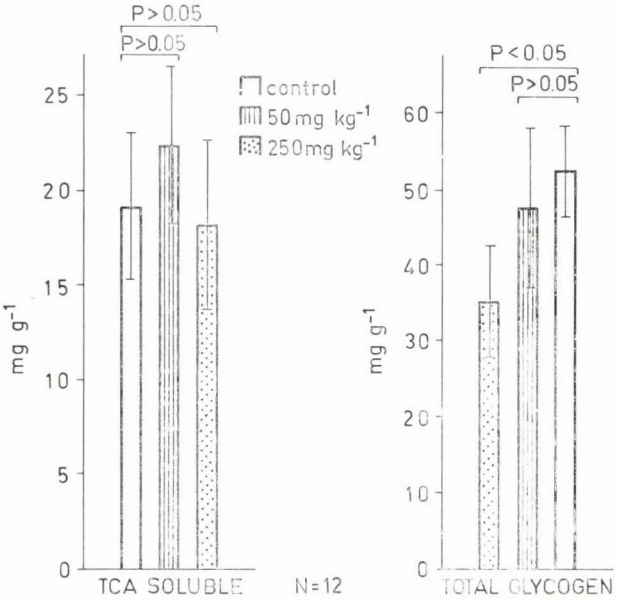


Fig. 3. Changes in the hepatic TCA-soluble and total glycogen concentrations after 30-day treatment with CH-401 (N = number of animals)

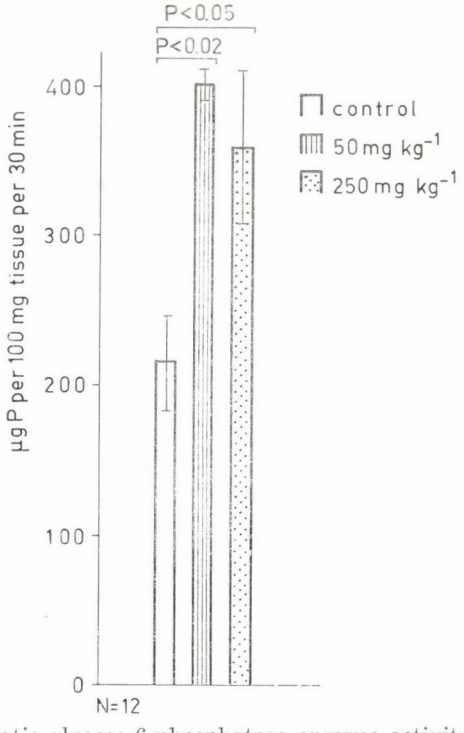


Fig. 4. Changes in hepatic glucose-6-phosphatase enzyme activity after treatment with CH-401 (N = number of animals)

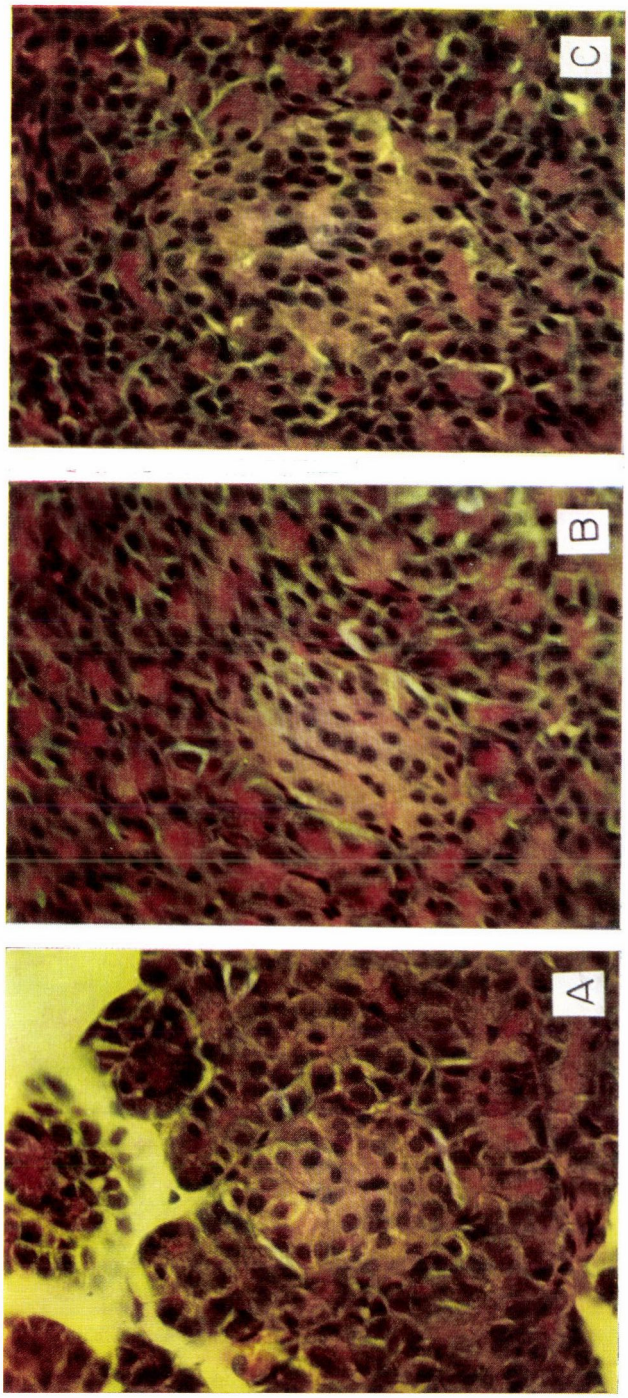


Fig. 5. Microscopic picture of the pancreas of CH-401-treated and control rabbits. A = control. B = 50 mg per kg bwt CH-401. C = 250 mg per kg CH-401. Magnification 12.5×40. Haematoxylin and chromotrop-$R_2$ staining after fixation in 4% neutral formalin

Changes in hepatic G-6-P-ase activity after 30 days on experimental diet are shown in Fig. 4. The CH-401 stimulated the activity of the enzyme significantly at both dose levels, but — although the functioning of this enzyme is the limiting factor of hepatic glucose metabolism — its increase in activity in a remarkable manner did not influence the blood glucose level. The cause of this anomalous phenomenon was not investigated.

Figure 5 shows the light micrographs of the pancreas of rabbits treated with the different doses of CH-401. At the lower level of treatment (50 mg per kg bwt) the exocrinic part of the gland remained intact; the islets of Langerhans were preserved, the insular cells stained well and showed the usual distribution. There was no indication of vacuolization or any other degenerative change. The insular cells of the rabbits treated with 250 mg per kg CH-401 also appeared preserved, except for a slight nuclear hyperplasia in a few cases. Tissular lesions indicative of diabetes were not found.

## 3. Discussion

The synthetic sweetener CH-401 was found to be highly active at organoleptic examination. As long-term treatment with CH-401 had no hyperglycaemic effect, the preparation seems to be suitable for use as sweetener in certain food items.

It should be noted that the CH-401 treated rabbits showed a conspicuous increase of the hepatic G-6-P-ase enzyme activity. This effect may have been due to the hepatic accumulation of the compound or its metabolites.

The decrease observed in the liver of bound glycogen concentration and the rise in G-6-P-ase enzyme activity call for an additional study of the pharmacological effect of the compound.

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Addresses of the authors:

Dr. Péter SZABÓ
Dr. Tibor FERENCZY
Department of Zoology and Anthropology,
Kossuth University
H-4010 Debrecen, Egyetem tér 1.
Hungary

Ms. Éva BOLLA-PUSZTAI
Dr. József SZEJTLI
Biochemical Research Laboratory,
Chinoin Pharmaceutical and Chemical Works
H-1026 Budapest, Endrődi S. u. 38–40.
Hungary

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# ALKYLRESORCINOLS IN DURUM WHEAT

## I. VARIETAL AND KERNEL QUALITY EFFECTS

H. SÁGI-LOMNICZI and F. SÁGI

(Received: 5 November 1980; accepted: 17 December 1980)

5-Alkylresorcinol (5-AR) content in kernels of fourteen durum wheat (*Triticum durum Desf.*) varieties was determined with special attention to kernel size, vitreousness and occurrence of blackpoints. An average 5-AR content of 0.075% dry weight was found with 0.108% and 0.046% as highest and lowest values, respectively. 5-AR content of vitreous and floury kernels did not differ significantly. Occurrence of blackpoints also did not influence the 5-AR level. However, the light, small kernels contained relatively more 5-AR than the heavier ones.

In recent years, the antinutritive 5-alkylresorcinol (5-AR) content of various cereal grains was thoroughly studied (MUNCK, 1972; EVANS *et al.*, 1973; STUCZYNSKI *et al.*, 1974; VERDEAL & LORENZ, 1977). Nevertheless, the maccaroni or durum wheat (*Triticum durum Desf.*) remained an exception in this respect. EVANS and co-workers (1973) reported that the *Stewart* 63 durum wheat contained 0.053% dry weight of 5-AR, and VERDEAL and LORENZ (1977) found 0.067% of 5-AR in a durum sample. No other data were accessible from the available literature. Therefore, it was decided to determine the 5-AR content of durum wheat on a wider varietal basis. The possible dependence of 5-AR content in durum wheats on some important kernel quality characteristics like vitreousness, occurrence of blackpoints and size was also investigated. Since there is an overall growing interest in production, breeding and utilization of durum wheat, this study seemed to be warranted.

### 1. Materials and methods

Seed samples of the *Durtal*, *Mondur* and *Valdur* varieties were obtained from the INSTITUT TECHNIQUE DES CÉRÉALES ET DES FOURRAGES, Paris (France). Seed samples of the other varieties of various origin were supplied by the WHEAT BREEDING DEPARTMENT of our Institute. All seeds were from the 1979 harvest. Vitreous and floury, normal and dotted, small and large kernels of certain varieties were selected by hand and used separately for 5-AR measurements.

Thirty unground, whole kernels were weighed in duplicate and extracted twice for 16 h each, with 10 $cm^3$ of acetone in a rotary shaker at room temperature. The extracts were combined, filtered and filled up to 30 $cm^3$ with acetone. Determination of the 5-AR content of extracts was then carried out by the diazotized *p*-nitraniline method of WEIPERT and EL BAYA (1977) using a *Spectromom* 361 visible spectrophotometer and a standard curve prepared with the main 5-AR component of durum wheat purified by silica gel G thin-layer chromatography ($R_f = 0.23$, see later). Dry matter contents of the kernels were determined by an usual oven method. All determinations were repeated at least five times. Significant differences were calculated according to the *t* test. For the chromatographic separation of 5-AR components, aliquots of the extracts were applied to 250 $\mu$m thin-layers of *Silicagel* G (MERCK). The chromatograms were run unidimensionally with chloroform–ethylacetate 95 : 5 (*v*/*v*) as solvent and the 5-AR spots were visualized by diazotized *p*-nitraniline.

Table 1

*5-AR content (% dry weight) of durum wheats. Determination in acetone extract by spectrophotometry at 435 nm after addition of diazotized* p*-nitralinine* (WEIPERT & EL BAYA, 1977)

| Variety | 5-AR (% dry weight) $\bar{x} \pm s$ |
|---|---|
| *Giorgio* | 0.108 ± 0.024 |
| *Mondur* | 0.091 ± 0.013 |
| *Stewart 63* | 0.087 ± 0.016 |
| *Valdur* | 0.087 ± 0.015 |
| *Raineri* | 0.082 ± 0.008 |
| *Agathe* | 0.081 ± 0.013 |
| *Durtal* | 0.081 ± 0.015 |
| *GK Basa* | 0.073 ± 0.011 |
| *Pepe 36* | 0.071 ± 0.010 |
| *Ringo* | 0.066 ± 0.009 |
| *Conte Morando* | 0.066 ± 0.014 |
| *Morando Bolognini* | 0.059 ± 0.012 |
| *GK Aga* | 0.058 ± 0.007 |
| *GK Minaret* | 0.046 ± 0.003 |
| Mean | 0.075 |

## 2. Results

As shown in Table 1, the 5-AR content in the kernels of fourteen examined durum wheats varied between 0.108% (*Giorgio*) and 0.046% dry weight (GK *Minaret*). Mean of the 5-AR content was 0.075% dry weight. On the thin-layer chromatograms, four major, low $R_f$-values (0.01, 0.05, 0.14, 0.23) and five minor, higher $R_f$-values diazotized *p*-nitraniline-reactive spots appeared (Fig. 1). The 5-AR components did not differ qualitatively; only slight quantitative differences were apparent between the varieties.

The 5-AR content of vitreous and floury kernels was determined in ten varieties. Vitreous or floury endosperm type did not influence the 5-AR content, except in the variety *Pepe* 36 (Table 2). However, in this case the difference was only weakly significant and the 5-AR content of the two kernel classes was practically identical in the average of all durum wheats involved in the experiment.

The 5-AR content of dotted and normal grains (Fig. 2) could be compared in four varieties. The results are summarized in Table 3. Again, there was no significant difference between the 5-AR content of dotted and normal grains.


Fig. 1. 5-AR components of durum wheat kernels. Layer: *Silicagel* G (MERCK), 250 μm. Solvent: chloroform–ethylacetate 95 : 5 (v/v). Spray reagent: diazotized *p*-nitraniline. 1: *Valdur*, 2: *Raineri*, 3: *Conte Morando*, 4: *Mondur*, 5: *Durtal*, 6: *GK Basa*

Table 2

*5-AR content in floury and vitreous kernels of several durum wheat varieties*

| Variety | 5-AR (% dry weight) | | |
|---|---|---|---|
| | floury kernel | vitreous kernel | difference |
| | $\bar{x} \pm s$ | $\bar{x} \pm s$ | |
| *Giorgio* | $0.104 \pm 0.021$ | $0.109 \pm 0.021$ | 0.005 |
| *Mondur* | $0.092 \pm 0.013$ | $0.090 \pm 0.010$ | 0.002 |
| *Stewart 63* | $0.085 \pm 0.011$ | $0.088 \pm 0.014$ | 0.003 |
| *Valdur* | $0.087 \pm 0.015$ | $0.083 \pm 0.012$ | 0.004 |
| *Raineri* | $0.085 \pm 0.010$ | $0.081 \pm 0.008$ | 0.004 |
| *Durtal* | $0.078 \pm 0.015$ | $0.084 \pm 0.013$ | 0.006 |
| *Agathe* | $0.077 \pm 0.006$ | $0.083 \pm 0.007$ | 0.006 |
| *GK Basa* | $0.070 \pm 0.008$ | $0.074 \pm 0.008$ | 0.004 |
| *Pepe 36* | $0.078 \pm 0.003$ | $0.067 \pm 0.009$ | 0.011* |
| *Conte Morando* | $0.066 \pm 0.008$ | $0.063 \pm 0.012$ | 0.003 |
| Mean | 0.082 | 0.082 | 0 |

* Significant at the $P \geq 5\%$ probability level

Fig. 2. Effect of fungal infection on durum wheat kernels. Variety: *Agathe*. Left: normal (healthy) kernels, right: dotted (infected) kernels

For comparison of the 5-AR level in the light and heavier kernels, twelve varieties were used. Table 4 demonstrates the thousand kernel weights and the related 5-AR contents of the two size groups as well as their corresponding ratios. As can be seen, there are overall highly significant differences between weights but not between the 5-AR contents in the two kernel groups. In addition, these differences are not in accordance with each other, since the

Table 3

*5-AR content of normal (healthy) and dotted (infected) durum wheat kernels*

| Variety | 5-AR (% dry weight) | | |
|---|---|---|---|
| | normal kernel | dotted kernel | difference |
| *Agathe* | 0.081 ± 0.013 | 0.085 ± 0.009 | 0.004 |
| *Conte Morando* | 0.066 ± 0.014 | 0.067 ± 0.010 | 0.001 |
| *Ringo* | 0.064 ± 0.009 | 0.067 ± 0.007 | 0.003 |
| *Morando Bolognini* | 0.059 ± 0.012 | 0.059 ± 0.012 | 0 |
| Mean | 0.067 | 0.069 | 0.002 |

Table 4

*Thousand kernel weight and 5-AR content in large and small kernels of durum wheats*

| Variety | Thousand kernel weight, (g) | | | 5-AR content, (μg/kernel+) | | |
|---|---|---|---|---|---|---|
| | Large (L) $\bar{x} \pm s$ | Small (S) $\bar{x} \pm s$ | *L/S* | Large (L) $\bar{x} \pm s$ | Small (S) $\bar{x} \pm s$ | *L/S* |
| *Giorgio* | 47.6 ± 3.0 | 24.1 ± 2.0*** | 1.97 | 47.2 ± 5.4 | 32.6 ± 3.1*** | 1.45 |
| *Valdur* | 54.3 ± 2.3 | 27.7 ± 2.1*** | 1.96 | 42.0 ± 0.8 | 29.8 ± 2.7** | 1.41 |
| *Mondur* | 50.7 ± 2.4 | 26.0 ± 2.4*** | 1.95 | 43.4 ± 6.3 | 30.8 ± 5.9* | 1.41 |
| *Stewart 63* | 53.5 ± 2.0 | 27.9 ± 2.6*** | 1.92 | 47.9 ± 1.5 | 31.4 ± 6.6** | 1.52 |
| *Pepe 36* | 50.9 ± 0.8 | 27.3 ± 2.6*** | 1.86 | 40.2 ± 4.8 | 27.3 ± 2.7** | 1.47 |
| *Agathe* | 54.7 ± 0.5 | 30.5 ± 2.2*** | 1.79 | 42.0 ± 1.4 | 25.9 ± 1.4*** | 1.62 |
| *Durtal* | 46.9 ± 2.0 | 27.0 ± 0.8*** | 1.74 | 35.0 ± 4.8 | 24.5 ± 3.5** | 1.43 |
| *Raineri* | 54.9 ± 1.6 | 32.8 ± 2.0*** | 1.67 | 41.6 ± 2.8 | 28.0 ± 4.9** | 1.48 |
| *GK Basa* | 51.1 ± 0.15 | 31.3 ± 1.6*** | 1.64 | 29.1 ± 2.8 | 24.8 ± 2.1 | 1.17 |
| *Ringo* | 55.3 ± 1.6 | 34.2 ± 2.3*** | 1.62 | 36.7 ± 2.8 | 25.9 ± 2.4** | 1.42 |
| *Conte Morando* | 52.6 ± 2.5 | 32.9 ± 3.2*** | 1.60 | 32.5 ± 3.4 | 23.0 ± 2.1* | 1.41 |
| *Morando Bolognini* | 49.4 ± 0.5 | 32.5 ± 0.8*** | 1.54 | 27.6 ± 4.5 | 24.5 ± 1.7 | 1.13 |
| Mean | 51.9 | 29.4** | 1.76 | 38.8 | 27.4** | 1.41 |

+ 10% moisture basis
* Significant at $P \leq 5\%$ probability level
** Highly significant at $P \leq 1\%$ probability level
*** Very highly significant at $P \leq 0.1\%$ probability level

thousand kernel weight ratios are consistently higher than the corresponding 5-AR ratios. Thus, light, small durum wheat grains are relatively richer in alkylresorcinols than their heavier counterparts.

## 3. Conclusions

Both range and average of 5-AR content in the kernels of the studied durum wheats (Table 1) are similar to those of bread wheats (700–1 050 mg per kg according to WEIPERT & EL BAYA, 1977) but our figures are generally higher than those reported so far for durum wheat in the literature (*i.e.* 0.053 and 0.067% dry matter, see Introduction). These discrepancies may be caused by the different extraction and determination procedures or by geographic variations, too. The 5-AR differences found between the varieties can probably be regarded as characteristic ones, since the grain 5-AR content seems to be independent of the crop year at the same location (VERDEAL & LORENZ, 1977), although the genetical mediation of these differences has not been verified yet.

The unidimensionally separated 5-AR components of durum wheat kernels on *Silicagel* G thin-layers show a very similar qualitative pattern (Fig. 1). This was also observed by MUSEHOLD (1978) in the case of bread wheat. Supposedly, durum wheats may possess the same genetical system for controlling 5-AR synthesis in their kernels. Lack of appropriate standards did not allow us to identify the major, low $R_f$-value of 5-AR components. However, since they are the most apolar, their alkyl side-chain might be composed at least of 19 to 25 C atoms (WIERINGA, 1967).

The higher the percentage of vitreous kernels in a durum wheat sample, the higher the semolina yield. Therefore, vitreousness is an extremely important quality parameter of durum wheat. Fortunately, the vitreous endosperm type is not associated with an elevated level of 5-AR (Table 2). This also holds true for the floury kernels, the proportion of which may rise up to 60% under some circumstances. It is probable that the 5-AR content of durum wheats is not affected by the kernel texture.

Deep brown or black points may develop occasionally on durum wheat grains as a result of fungal infection (Fig. 2). The varieties are not equally sensitive to this grain damage, and incidence of blackpoints also changes year to year. If present, the dotted grains must be removed carefully from the durum samples before milling, otherwise they render the manufactured pasta products (macaroni, spaghetti, *etc.*) also dotted and of no commercial value. Consequently, 5-AR content of dotted kernels is indifferent from the aspect of human nutrition. However, for the durum wheat as a host plant, this may not be the case, as it is well known that fungal attack generally activates the phenolic biosynthesis and leads to an accumulation of polyphenols in a number of plant tissues (FARKAS & KIRÁLY, 1962). But, contrary to expectation, the 5-AR content of the diseased (dotted) durum wheat kernels did not increase as compared to that of the normal controls (Table 3). Apparently, net synthesis of these phenolics is not affected by the infection, or, alternatively, poly-

phenol metabolism of the kernels has already become non-responsive at the infection time owing to some physiological alterations during maturation.

Independently of the variety, there was relatively more 5-AR in the small than in the large durum wheat kernels (Table 4). STUCZYNSKI and co-workers (1974) have already called attention to this phenomenon when studying the 5-AR content of various cereals (except durum wheat). Results of WEIPERT and EL BAYA (1977) with wheats and rye confirm the finding of the former authors. This can be explained by the relatively greater proportion of pericarp in the smaller kernels. As stated by WIERINGA (1967), WEIPERT and EL BAYA (1977) and others, 5-alkylresorcinols mainly concentrate in the pericarp of cereal grains. Thus, it is not surprising that the 5-AR content increases with decreasing thousand kernel weight in durum wheat, too. This relationship underlines the importance of sorting out the small kernels from durum seedlots during cleaning. For the same reason, breeding and growing of durum wheats of uniform kernel size would also be desirable.

*

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Address of the authors:

Ms. Hajnalka SÁGI-LOMNICZI
Dr. Ferenc SÁGI

Cereal Research Institute
H-6701 Szeged, Alsókikötősor 9.
Hungary

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# ATTEMPTS TO DETERMINE EGG CONTENT IN PASTRY PRODUCTS USING THE NIR TECHNIQUE

K. J. KAFFKA and F. KULCSÁR

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Preliminary studies were made on pastry samples produced without eggs and with two, four and six eggs per kg, resp., in order to establish how composition parameters can be determined with reflection measurements in the near infrared wavelength region and to estimate the accuracy in predicting the composition parameters of pastry products using the instrumental method mentioned.

A *Neotec* 6450 type Research Composition Analyzer — a computerized spectrophotometer — was used to study the 25 pastry samples produced in the CANNING FACTORY, Békéscsaba on a *Braibanti* production line. The pastry samples were labelled with the composition parameters determined by traditional standard methods at the COUNTY INSTITUTE FOR FOOD CONTROL AND CHEMISTRY, Békéscsaba. These parameters were: egg content per kg pastry, fat content, protein content and moisture content in mass %.

The relationships between composition parameters and optical properties were determined for the 25 pastry samples by regression analysis. It was possible to predict egg content with a standard error of estimate of 0.166 (egg content per kg pastry) with reflection measurements at the wavelengths of 1650 and 1724 nm. The fat content could be predicted with a standard error of estimate of 0.006 (mass %) from spectral data measured at the same above mentioned wavelengths. The protein content showed a standard error of estimate of 0.067 (mass %) predicted from measurements at the same wavelengths.

From the fact that with the *Neotec* RCA type 6450 the same characteristic wavelengths were found for both egg and protein content, we deduced that the determination of the composition of pastry products was based on fat. The two characteristic wavelengths represented the local maximum and minimum, resp., of the reflection spectrum of fat. This is, however, true because, by changing the egg content, the fat and protein contents vary at the same rate but the percentual fat content changes more significantly than does the protein content. Using the first derivative of reflection spectra resulted in higher accuracy.

Further experiments are to be performed for determining the fat and protein content separately in pastry samples where fat and protein content vary independently. Preparation has already been made for this.

The above described studies lead to the conclusion that in the case of pastry products, the egg content can be predicted by a quick, accurate and non-destructive instrumental method using near-infrared reflection data measured at some characteristic wavelengths.

The ever increasing demand for constant and improved quality of food products required the development of instruments for the rapid determination of quality parameters.

The most significant quality parameters of pastry products are egg, fat, protein and water contents. The analytical methods for determining the above parameters are specified in the respective HUNGARIAN STANDARDS (1972c, 1979a, 1980). The time required for these chemical analyses is too long to base

the automatic control of production on them. On the other hand, the time and working power requirements also hinder the application of these methods for the control of quality of pastry products in the shops.

For determining the composition of raw materials used in pastry production the NIR (near-infrared) technique has already been successfully applied. Fat, protein, water, *etc.* content could be determined in these raw materials using the values of reflection data measured at different characteristic wavelengths in the near infrared range.

MERCURI and co-workers (1957) published a paper on an instrument using NIR techniques for determining the quality of liquid egg, performing measurements at two characteristic wavelengths.

NORRIS and ROWAN (1962) presented an instrument which could detect blood in eggs using NIR technique and so open the possibility of automatic sorting.

ROSENTHAL (1973) mentioned an instrument to determine moisture, oil and protein content rapidly and accurately in grain and grain products by means of the NIR technique.

STERMER and co-workers (1977) gave an account of their achievements in estimation of moisture in whole grain using the NIR technique.

WILLIAMS and co-workers (1978) and BOLLING (1979) used the NIR technique for testing wheat for protein and moisture.

MILLER and POMERANZ (1978) dealt with the comparison of *Kjeldahl* and NIR procedures to see the interlaboratory and intralaboratory reproducibility of protein determination.

WILLIAMS and THOMPSON (1978) studied the influence of whole meal granularity (particle size distribution) on the accuracy of protein and water determination in wheat using the NIR technique.

On the basis of the above results, it was promising to study the applicability of this technique for determining the composition of pastry products So far such measurements have not yet been reported.

There are two main measuring instrument producers that provide instruments for the NIR technique. In an 1978 *Sales News* leaflet NEOTEC mentioned 50 products where the NIR technique was successfully employed whereas TECHNICON already suggested to use this technique for 82 products in a leaflet at ACHEMA '79. Neither list contained pastry products.

This preliminary study aims at finding the possibility for applying the NIR technique for the rapid determination of egg, fat and protein content in pastry products; and at the same time to predict accuracy for the different components, to choose the most suitable form of the regression equations, determining the parameters (characteristic wavelengths, coefficients and constants) and last but not least to examine the achievable accuracy by transformation studies on the reflection spectra. The results of these studies will

hopefully enable us to develop a single-purpose instrument or to extend the usage of another one to be able to perform such measurements.

The timeliness of our research work was stressed by the fact, that, in order to modify and to modernize the respective items of HUNGARIAN STANDARD (1972c), a multiple examination was carried out organized by the CENTRE OF FOOD CONTROL AND ANALYSIS OF THE MINISTRY OF AGRICULTURE AND FOOD, Budapest and the COUNTY INSTITUTE FOR FOOD CONTROL AND CHEMISTRY, Békéscsaba to compare the methods used so far for determining the egg content in pastry products.

## 1. Materials and methods

Pastry products (sometimes also called pastas) are made of flour and water with or without eggs by mixing, kneading, rolling or forming, resp., then dried ready for cooking.

The relevant HUNGARIAN STANDARDS for pastry products are as follows: Classification, denomination and technical demand for different pastry products (1979a); Sampling and quality assessment of pastry products (1980); Test methods — physical properties (1972b); Test methods — chemical properties (1972c); Test methods — sensory properties (1979b); Fat determination — *Stoldt-Weibull* method (1979c).

For the production of pastries, the two most important raw materials are flour and egg. The HUNGARIAN STANDARDS for these are as follows: Wheat flour for pastry products (1968, 1972a); Eggs quality requirements (1978b); Egg powder quality requirements (1977a); Egg powder sampling, examination, quality assessment (1977b).

Water and salt are also needed and the respective HUNGARIAN STANDARDS are as follows: Water quality (1978c); Salt quality (1978a). The pastry samples used for our examination were made of TL 50 flour corresponding to HUNGARIAN STANDARD (1968). This means an ash content less than 0.5 mass %, wet gluten minimum 28 mass %, moisture content less than 15.2 mass %, particle size between 200–400 $\mu$m (maximum 15% below 200 $\mu$m). The egg powder used corresponded to the requirements of HUNGARIAN STANDARD (1977a). The pastries were made in the CANNING FACTORY, Békéscsaba on a *Braibanti* production line. Samples were prepared at the COUNTY INSTITUTE FOR FOOD CONTROL AND ANALYSIS, Békéscsaba according to HUNGARIAN STANDARD (1972c). The egg content of the respective pastry samples was guaranteed by the COUNTY INSTITUTE FOR FOOD CONTROL AND ANALYSIS, Békéscsaba (egg and flour rates were carefully monitored). Fat content was determined by the *Stoldt–Weibull* method, whereas protein content was determined with the *Kjel–Foss* instrument.

Our examinations were performed on 25 pastry samples well corresponding to market demands, *i.e.* without egg, with 2, 4 and 6 eggs; meaning that one kg of dried pastry product contained egg powder representing 0, 2, 4 or 6 eggs, resp.

The optical properties of pastry samples were measured with a 6450 type *Neotec* Research Composition Analyzer (RCA) in the reflection mode.

We placed 10 $cm^3$ of powdered pastry sample into the sample holder of 54 mm diameter bordered with a 2 mm thick glass plate on one side and pressed with a pressure of about 0.1 mPa from the other side. Illumination was provided with a beam of 25 mm diameter at an incident angle of 90° through the glass plate (the illumination was perpendicular to the surface of the sample). Sensors were placed to measure the beam reflected at 45°. The spectro-analyzer was set to measure in the 1 100–2 500 nm wavelength range; spectra were measured with 10 nm spectral bandpass in 2 nm stepts.

The reflection spectra were formed as the negative logarithm of the reflectometer value:

$$-\log R' = \log \frac{1}{R'}$$

where the reflectometer value

$$R' = \frac{I_{\text{sample}}}{I_{\text{etalon}}}$$

is the quotient of the energy of the incident beam reflected on the detector from the sample ($I_{\text{sample}}$) and from the ceramic etalon ($I_{\text{etalon}}$).

The resolution of the measurement was 0.0003 $\log \frac{1}{R'}$ unit (1 $\log \frac{1}{R'}$ unit corresponds to 3 276 steps).

Each reflection spectrum of a sample was taken as the average of 50 scannings (the whole operation took 20 s). The samples were re-loaded into the sample holder 5 times and measurement was subsequently repeated 5 times and averages of the spectra thus obtained were stored on floppy-discs for evaluation and further processing. The spectra were stored together with the related data on the composition parameters of the samples. The RCA type 6 450 is suitable for the automatic computation of the correlation between the reflection data measured at different wavelengths and the compositional data. It determines the wavelength — where the correlation reaches its peak and records the coefficients and constants of the regression equation together with the standard error of estimate and the $F$ value. The procedure is repeatable in order to determine the second, third and even further characteristic wavelength with the same method. The equipment can also transform the spectra prior to data processing so that the information content of significant interest

can be emphasized, concentrated and at the same time disturbing features depressed (*e.g.* changes in thickness, differences in distribution of particle sizes, temperature changes, *etc.*).

The procedure of obtaining the equation — which can have different forms — giving the best correlation is performed by multiple regression analysis that gives the quality parameter as a multivariable function of reflection data measured at the characteristic wavelengths with highest accuracy.

The definition of the standard error of estimate is

$$\sqrt{\frac{\sum_{i=1}^{n} (Q_{si} - Q_{ci})^2}{n - 1 - p}}$$

where $n$ is the number of samples, $p$ is the number of independent variables, $Q_s$ is the respective quality parameter determined by standard methods, $Q_c$ is the same quality parameter computed from the regression equation.

## 2. Results

The compositional data — namely egg, fat and protein content — of 25 pastry samples are summarized in Table 1. The moisture content of each sample was adjusted to 10.6 mass %.

We randomly selected four samples from the lot in order to have one sample each for pastry made with 0, 2, 4, 6 eggs. The reflection spectra of these four samples are seen in Fig. 1.

From the spectra it can be noticed that the higher the egg content the lower the $\log \frac{1}{R'}$ values; that is the reflected energy increases along with the increase in egg content.

The reproducibility of spectra was determined by repeated measurements, the same pastry sample was re-loaded into the sample holder and re-measured in the spectro-analyzer 5 times. Reproducibility was characterized by the standard deviation of the spectral data the values of which can be seen in Fig. 2 as a function of wavelength.

The Figure illustrates that, apart from a few narrow wavebands, the scatter of spectra is around 0.001 $\log \frac{1}{R'}$ unit.

In Fig. 3 the differences between the spectra of pastry samples of different egg content — see Fig. 1 — referred to that without eggs can be seen.

The RCA type 6450 instrument sought correlation between the $\log 1/R'$, values and egg, fat and protein content determined with standard methods on 25 pastry samples using different forms of equation.

Table 1

*Composition of the 25 pastry samples*

| Sample No. | Number of eggs per kg pastry | Fat content (weight %) | Protein content (weight %) |
|---|---|---|---|
| 1 | 0 | 1.37 | 11.92 |
| 2 | 6 | 3.89 | 13.76 |
| 3 | 4 | 2.92 | 13.05 |
| 4 | 0 | 1.37 | 11.95 |
| 5 | 2 | 1.98 | 12.40 |
| 6 | 0 | 1.37 | 11.85 |
| 7 | 4 | 2.92 | 13.04 |
| 8 | 6 | 3.89 | 13.79 |
| 9 | 0 | 1.37 | 11.90 |
| 10 | 0 | 1.37 | 11.90 |
| 11 | 6 | 3.89 | 13.75 |
| 12 | 2 | 1.98 | 12.35 |
| 13 | 4 | 2.92 | 13.10 |
| 14 | 0 | 1.37 | 11.85 |
| 15 | 6 | 3.89 | 13.75 |
| 16 | 6 | 3.89 | 13.90 |
| 17 | 2 | 1.98 | 12.40 |
| 18 | 6 | 3.89 | 13.65 |
| 19 | 2 | 1.98 | 12.40 |
| 20 | 4 | 2.92 | 13.25 |
| 21 | 4 | 2.92 | 13.05 |
| 22 | 2 | 1.98 | 12.40 |
| 23 | 2 | 1.98 | 12.40 |
| 24 | 4 | 2.92 | 13.25 |
| 25 | 6 | 3.89 | 13.85 |

Correlation was first studied using equations of the following forms:

$$Q_1 = K_0 + K_1 V_{\lambda 1} \tag{1}$$

$$Q_2 = K_2 + K_3 V_{\lambda 1} + K_4 V_{\lambda 2} \tag{2}$$

$$Q_3 = K_5 + K_6 V_{\lambda 1} + K_7 V_{\lambda 2} + K_8 V_{\lambda 3} \tag{3}$$

where $Q_1$, $Q_2$, $Q_3$ stand for composition parameters; $K_0 \ldots K_8$, are coefficients

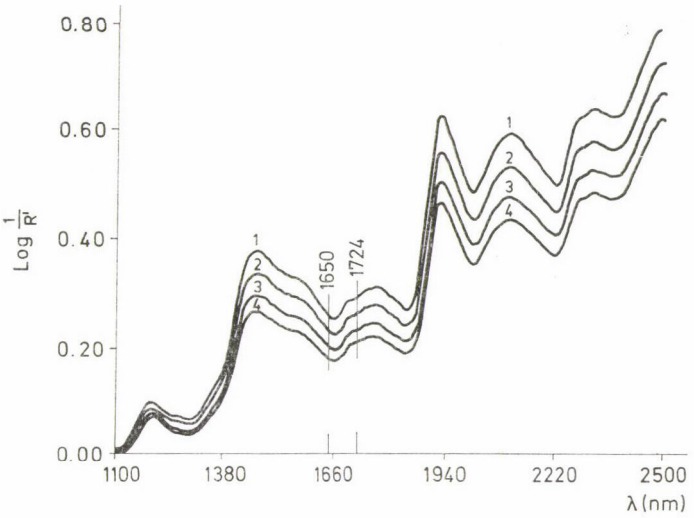


Fig. 1. Reflection spectra of pastry samples. The measuring geometry: 90°/45°. Diameter of the illuminating beam: 25 mm. Spectral bandpass: 10 nm. Spectra were taken in 2 nm steps. Vertical resolution: 0.0003 $\log \frac{1}{R'}$ unit

1: Pastry sample No. 4 made without eggs; 2: Pastry sample No. 12 made with two eggs per kg pastry; 3: Pastry sample No. 13 made with four eggs per kg pastry; 4: Pastry sample No. 2 made with six eggs per kg pastry $R' = \frac{I_{\text{sample}}}{I_{\text{etalon}}}$

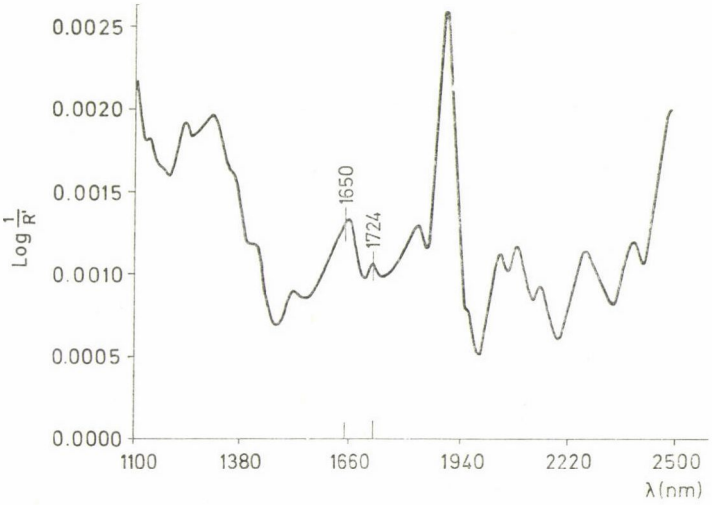


Fig. 2. Reproducibility of reflection spectra of pastry samples characterized by standard deviation of the values of repeated spectral data as a function of wavelengths. The measuring geometry: 90°/45°. Spectral bandpass: 10 nm. Spectra were taken in 2 nm steps

$$R' = \frac{I_{\text{sample}}}{I_{\text{etalon}}}$$

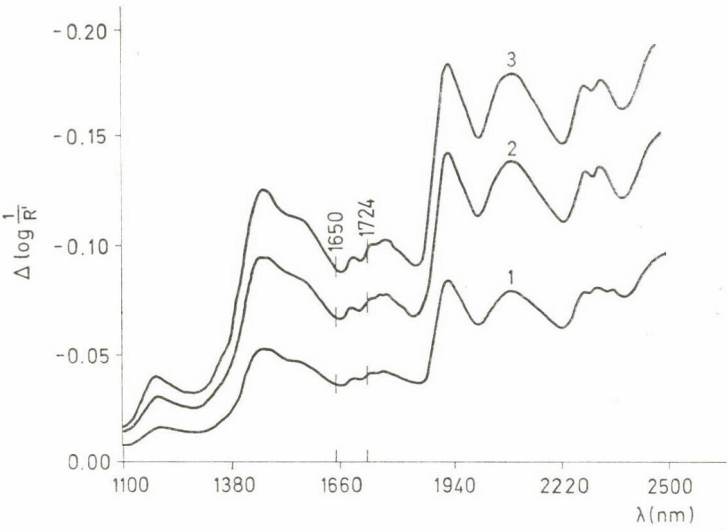


Fig. 3. The differences among the reflection spectra shown in Fig. 1 for pastry samples of different egg content referred to that without eggs. 1: $\Delta$ spectrum for pastry sample No. 12 made with 2 eggs; 2: $\Delta$ spectrum for pastry sample No. 13 made with 4 eggs; 3: $\Delta$ spectrum for pastry sample No. 2 made with 6 eggs $R' = \frac{I_{\text{sample}}}{I_{\text{etalon}}}$

and constants, resp.; $\lambda_1$, $\lambda_2$, $\lambda_3$ are characteristic wavelengths; $V_{\lambda 1}$, $V_{\lambda 2}$ and $V_{\lambda 3}$ are the log $\frac{1}{R'}$ values belonging to these characteristic wavelengths.

Trying to find the first characteristic wavelength for egg content, the analyzer first determined the correlation between the egg content of pastry samples and the log $\frac{1}{R'}$ values measured at 2 nm steps in the 1 100–2 500 nm wavelength range. The curve of correlation coefficients belonging to different wavelengths is seen in Fig. 4.

The equipment marked the first characteristic wavelength, where the curve had its peak.

Following this, the apparatus determined the second and third characteristic wavelengths for egg content in a similar way assuming the former ones as fixed.

Determination of the wavelengths characteristic for fat and protein content was performed by the same method.

The results are summarized in Table 2 containing the characteristic wavelengths, the coefficients and constants of regression equations, standard error of estimate, correlation coefficients as well as the $F$ values.

It can be seen that by increasing the number of characteristic wavelengths — used in the regression equations — accuracy generally improves.

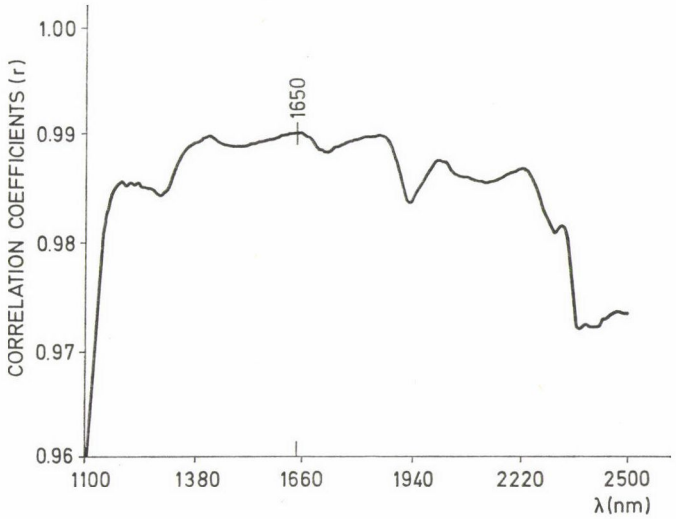


Fig. 4. The curve of correlation coefficients ($r$) which characterize the relationship between egg content of the pastry samples and $\log \frac{1}{R'}$ values measured at different wavelengths

The analyzer graphically displayed composition values determined by standard methods versus data computed from Equations No. 1, No. 2 and No. 3 resp., as well as the regression line.

As an example, the correlations between data determined by standard method and regression equation No. 1 for egg, fat and protein content are shown in Figs. 5, 6 and 7 while the same can be seen in Figs. 8, 9 and 10 using regression equation No. 2.

After these studies with the above three equation forms, we tried to find correlation between composition data of pastry samples and $\log \frac{1}{R'}$ values measured at different wavelengths using the following equation forms:

$$Q_4 = K_9 + K_{10} \frac{V_{\lambda 4}}{V_{\lambda 5}} \tag{4}$$

$$Q_5 = K_{11} + K_{12} \frac{V_{\lambda 4}}{V_{\lambda 5}} + V_{\lambda 6} \tag{5}$$

Wavelengths characteristic for egg, fat and protein content, the coefficients and constants of the regression equations, standard error of estimate, correlation coefficients as well as $F$ values were determined by the above described method the results of which are summarized in Table 3.

Table 2

*Correlation between egg, fat and protein content of pastry samples and log $\frac{1}{R}$, values measured at one, two and three characteristic wavelengths*

| Equation form $Q_1 = K_0 + K_1 \nabla\lambda_1$ | | | |
|---|---|---|---|
| | for egg content | for fat content | for protein content |
| Characteristic wavelength $\lambda_1$ (nm) | 1 650 | 1 650 | 1 650 |
| Constant $K_0$ | 16.934 | 8.431 | 17.179 |
| Coefficient $K_1$ | −62.240 | −26.300 | −19.594 |
| Standard error of estimate | 0.331 | 0.222 | 0.149 |
| Correlation coefficient | 0.990 | 0.976 | 0.980 |
| $F$ test | 1 148 | 458.7 | 560.8 |
| Equation form $Q_2 = K_2 + K_3 \nabla\lambda_1 + K_4 \nabla\lambda_2$ | | | |
| | for egg content | for fat content | for protein content |
| Characteristic wavelength $\lambda_1$ (nm) | 1 650 | 1 650 | 1 650 |
| Characteristic wavelength $\lambda_2$ (nm) | 1 724 | 1 724 | 1 724 |
| Constant $K_2$ | 3.767 | −1.278 | 11.220 |
| Coefficient $K_3$ | −584.679 | −411.526 | −256.050 |
| Coefficient $K_4$ | 495.279 | 365.199 | 224.164 |
| Standard error of estimate | 0.166 | 0.006 | 0.067 |
| Multiple correlation coefficient | 0.998 | 0.998 | 0.996 |
| $F$ test | 69.7 | 294.6 | 90.6 |
| Equation form $Q_3 = K_5 + K_6 \nabla\lambda_1 + K_7 \nabla\lambda_2 + K_8 \nabla\lambda_3$ | | | |
| | for egg content | for fat content | for protein content |
| Characteristic wavelength $\lambda_1$ (nm) | 1 650 | 1 650 | 1 650 |
| Characteristic wavelength $\lambda_2$ (nm) | 1 724 | 1 724 | 1 724 |
| Characteristic wavelength $\lambda_3$ (nm) | 1 980 | 1 428 | 1 438 |
| Constant $K_5$ | 2.012 | −1.071 | 11.095 |
| Coefficient $K_6$ | −887.244 | −350.419 | −223.529 |
| Coefficient $K_7$ | 1,026.410 | 433.300 | 303.622 |
| Coefficient $K_8$ | −145.888 | −104.601 | −86.912 |
| Standard error of estimate | 0.111 | 0.045 | 0.059 |
| Multiple correlation coefficient | 0.999 | 0.999 | 0.997 |
| $F$ test | 28.5 | 17.6 | 8.2 |

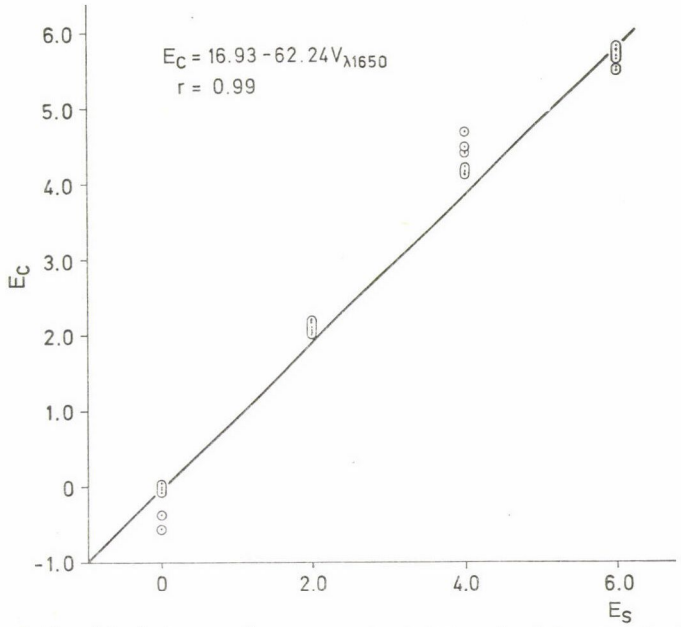


Fig. 5. The relationship between the egg content determined by standard method ($E_s$) and the egg content computed from regression equation ($E_c$) form No. 1. $E_s$: egg content per kg pastry determined by standard method; $E_c$: egg content per kg pastry calculated from NIR data

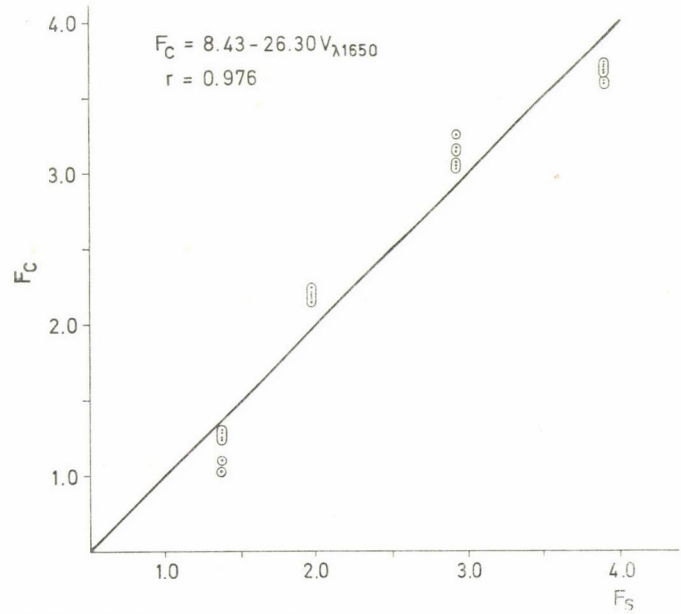


Fig. 6. The relationship between the fat content determined by standard method ($F_s$) and the fat content computed from regression equation ($F_c$) form No. 1. $F_s$: fat content in mass % determined by standard method; $F_c$: fat content in mass % calculated from NIR data

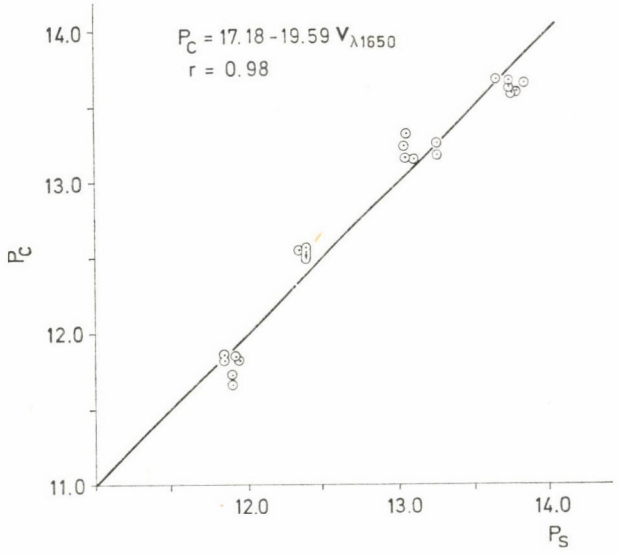


Fig. 7. The relationship between the protein content determined by standard method ($P_s$) and the protein content computed from regression equation ($P_c$) form No. 1. $P_s$: protein content in mass % determined by standard method; $P_c$: protein content in mass % calculated from NIR data

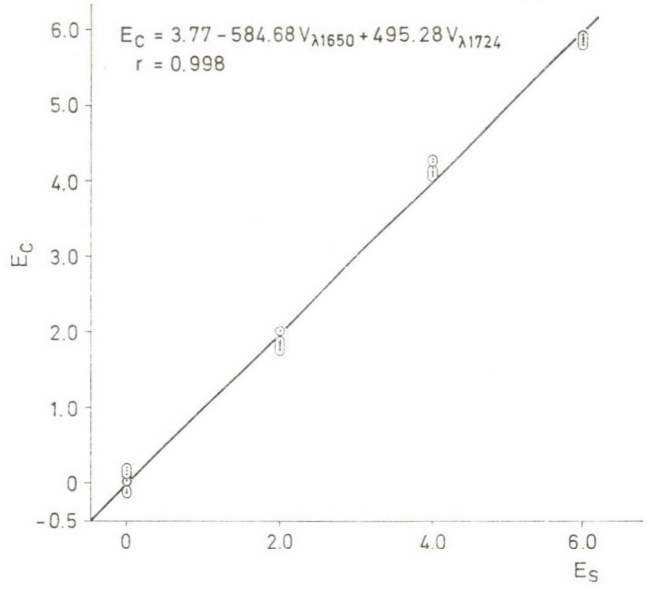


Fig. 8. The relationship between the egg content determined by standard method ($E_s$) and the egg content computed from regression equation ($E_c$) form No. 2. $E_s$: egg content per kg pastry determined by standard method; $E_c$: egg content per kg pastry calculated from NIR data

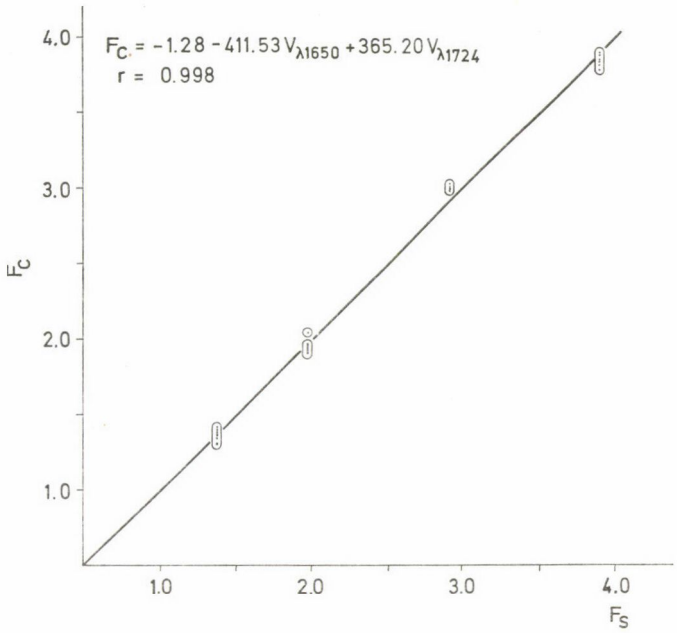


Fig. 9. The relationship between the fat content determined by standard method ($F_s$) and the fat content computed from regression equation ($F_c$) form No. 2. $F_s$: fat content in mass % determined by standard method; $F_c$: fat content in mass % calculated from NIR data

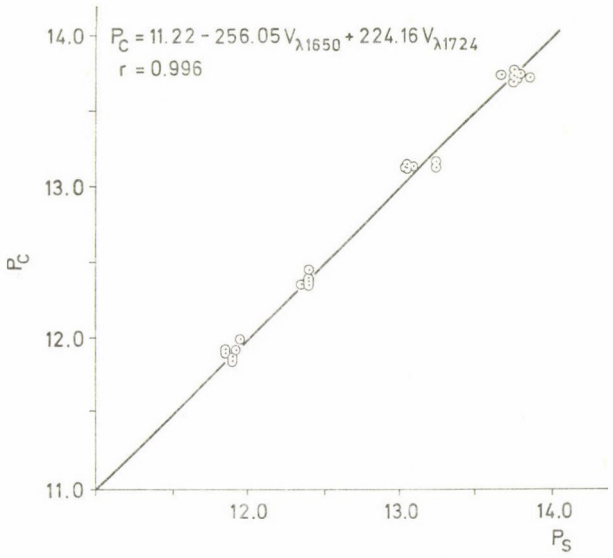


Fig. 10. The relationship between the protein content determined by standard method ($P_s$) and protein content computed from regression equation ($P_c$) form No. 2. $P_s$: protein content in mass % determined by standard method; $P_c$: protein content in mass % calculated from NIR data

Table 3

*Correlation between egg, fat and protein content of pastry samples and log $\frac{1}{R'}$ values measured at one, two and three characteristic wavelengths*

| Equation form $Q_4 = K_9 + K_{10}\frac{V\lambda_4}{V\lambda_5}$ | | | |
|---|---|---|---|
| | for egg content | for fat content | for protein content |
| Characteristic wavelength $\lambda_4$ (nm) | 1 650 | 1 650 | 1 650 |
| Characteristic wavelength $\lambda_5$ (nm) | 1 824 | 1 720 | 1 824 |
| Constant $K_9$ | 224.420 | 59.115 | 82.994 |
| Coefficient $K_{10}$ | −247.267 | −66.106 | −78.397 |
| Standard error of estimate | 0.203 | 0.044 | 0.074 |
| Multiple correlation coefficient | 0.996 | 0.999 | 0.995 |
| $F$ test | 3 087.1 | 12 391.8 | 2 357.2 |

| Equation form $Q_5 = K_{11} + K_{12}\frac{V\lambda_4}{V\lambda_5} + K_{13}V\lambda_6$ | | | |
|---|---|---|---|
| | for egg content | for fat content | for protein content |
| Characteristic wavelength $\lambda_4$ (nm) | 1 650 | 1 650 | 1 650 |
| Characteristic wavelength $\lambda_5$ (nm) | 1 824 | 1 720 | 1 824 |
| Characteristic wavelength $\lambda_6$ (nm) | 2 356 | 1 104 | 2 440 |
| Constant $K_{11}$ | 176.582 | 61.016 | 81.636 |
| Coefficient $K_{12}$ | −188.683 | −68.303 | −76.728 |
| Coefficient $K_{13}$ | −8.022 | 5.607 | −0.202 |
| Standard error of estimate | 0.185 | 0.045 | 0.076 |
| Multiple correlation coefficient | 0.997 | 0.999 | 0.995 |
| $F$ test | −8.022 | −1.1 | −0.6 |

Following this, we endeavoured to increase accuracy by transforming $\log\frac{1}{R'}$ spectra. Thus the first derivative of the $\log\frac{1}{R'}$ spectra of the 25 pastry samples was produced in the analyzer, then treating these spectra in the above-mentioned manner, correlation between transformed spectral data and composition parameters were studied in the following forms of equations:

$$Q_6 = K_{14} + K_{15}V^*_\lambda \tag{6}$$

$$Q_7 = K_{16} + K_{17}V^*_{\lambda 7} + K_{18}V^*_{\lambda 8} \tag{7}$$

$$Q_8 = K_{19} + K_{20}V^*_{\lambda 7} + K_{21}V^*_{\lambda 8} + K_{22}V^*_{\lambda 9} \tag{8}$$

Table 4

*Correlation between egg, fat and protein content of pastry samples and the values of the first derivative of the log $\frac{1}{R}$, spectra determined at one, two and three characteristic wavelengths*

Equation form $Q_6 = K_{14} + K_{15} \nabla^*_{\lambda 7}$

| | for egg content | for fat content | for protein content |
|---|---|---|---|
| Characteristic wavelength $\hat{\lambda}_7$ (nm) | 1 738 | 1 770 | 1 770 |
| Constant $K_{14}$ | 13.723 | 1.382 | 11.928 |
| Coefficient $K_{15}$ | 1 276.358 | 1 842.439 | 1 381.114 |
| Standard error of estimate | 0.192 | 0.104 | 0.084 |
| Correlation coefficient | 0.997 | 0.995 | 0.994 |
| $F$ test | 3 477.6 | 2 173.5 | 1 862.0 |

Equation form $Q_7 = K_{16} + K_{17} \nabla^*_{\lambda 7} + K_{18} \nabla^*_{\lambda 8}$

| | for egg content | for fat content | for protein content |
|---|---|---|---|
| Characteristic wavelength $\lambda_7$ (nm) | 1 738 | 1 770 | 1 770 |
| Characteristic wavelength $\lambda_8$ (nm) | 1 688 | 1 848 | 2 106 |
| Constant $K_{16}$ | 7.053 | 2.471 | 13.002 |
| Coefficient $K_{17}$ | 1,687.318 | 1,681.127 | 933.026 |
| Coefficient $K_{18}$ | −454.157 | 558.687 | −517.473 |
| Standard error of estimate | 0.085 | 0.085 | 0.070 |
| Multiple correlation coefficient | 0.999 | 0.997 | 0.996 |
| $F$ test | 93.9 | 12.3 | 10.4 |

Equation form $Q_8 = K_{19} + K_7 \nabla^*_{\lambda 7} + K_{21} \nabla^*_{\lambda 8} + K_{22} \nabla^*_{\lambda 9}$

| | for egg content | for fat content | for protein content |
|---|---|---|---|
| Characteristic wavelength $\lambda_7$ (nm) | 1 738 | 1,770 | 1 770 |
| Characteristic wavelength $\lambda_8$ (nm) | 1 688 | 1,848 | 2 106 |
| Characteristic wavelength $\lambda_9$ (nm) | 2 398 | 1,662 | 1 846 |
| Constant $K_{19}$ | 5.616 | 1.423 | 12.621 |
| Coefficient $K_{20}$ | 1 721.866 | 912.286 | 1 029.192 |
| Coefficient $K_{21}$ | −659.749 | 868.011 | −594.568 |
| Coefficient $K_{22}$ | 108.862 | − 1 096.236 | 459.569 |
| Standard error of estimate | 0.051 | 0.037 | 0.055 |
| Multiple correlation coefficient | 1.000 | 0.999 | 0.998 |
| $F$ test | 40.8 | 92.3 | 15.2 |

Where $V^*_{\lambda 7}$, $V^*_{\lambda 8}$ and $V^*_{\lambda 9}$ are the values of the first derivative of the $\log \frac{1}{R'}$ spectra at $\lambda_7$, $\lambda_8$ and $\lambda_9$ characteristic wavelengths.

Characteristic wavelengths, coefficients and constants of regression equations, standard error of estimate, correlation coefficients as well as $F$ values were determined using the method described. Results are summarized in Table 4.

## 3. Conclusions

It was clearly demonstrated that, under the above conditions, the NIR technique is very suitable for rapid instrumental determination of egg content in pastries. This can be stated despite the fact that our studies performed on pastry products in the near infrared wavelength range can only be considered as preliminary because we tried to avoid all disturbing influences as far as possible.

Before drawing final conclusions, attention must be called to the circumstances which open up the possibility of achieving these results. These circumstances are difficult to reach in every-day practice; *viz.* all pastry samples were produced from the very same flour; the pastry samples of different egg content contained the same homogenized egg powder; moisture was carefully adjusted before each measurement; samples were ground in the same mill under identical circumstances and were put through the same sieve to obtain identical distribution of particle size; the samples obtained in this way were filled into sample holders using the same pressure; temperature was kept constant; five parallel measurements were performed and averaged in order to increase accuracy.

However optimistic the above results may appear, it is proved how suitable the NIR technique is for determining the egg content provided disturbing effects are excluded or compensated. Compensation can be achieved by taking into account wavelengths characteristic for these disturbing effects and incorporating them into the regression equation.

Our experiments proved that satisfactory accuracy is obtained even if only two characteristic wavelengths are used for the determination of the egg content. A linear equation provided a slightly better result than the equation containing fractions.

Far better accuracy was obtained with the transformation of $\log \frac{1}{R'}$ spectra, namely by using the derivative of the spectra.

It was most surprising to find that characteristic wavelengths for determining egg, fat and protein content — independently of the form of the regression equation — were almost identical. This will easily be understood,

if we take into consideration that increasing egg content, fat and protein content increase at the same rate.

By studying the two characteristic wavelengths, we recognized that one of the characteristic wavelengths corresponded to the peak (local maximum) of fat spectrum whereas the other corresponded to that of the low point (local minimum) of both fat and protein spectra.

The regression equation of different forms given in this preliminary study determines egg content as well as protein content on the basis of the optical characteristics of fats. Thus the accuracy of data for protein content are to be viewed with certain reservation.

If we want to determine by the NIR technique fat and protein content independently, and also to estimate the accuracy of protein determination, the same studies must be repeated with pastry samples where fat and protein content vary independently. These tests are under way.

*

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Addresses of the authors:

Dr. Károly J. KAFFKA — Central Food Research Institute
H-1022 Budapest, Herman Ottó út 15.
Hungary

Dr. Ferenc KULCSÁR — Centre of Food Control and Analysis of the Ministry of Agriculture and Food
H-1051 Budapest, Guszev utca 25.
Hungary

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# HEAT-INDUCED CHANGES IN THE TEXTURE OF CARROTS

M. HORVÁTH-MOSONYI and L. HORVÁTH

(Received: 14 November 1980; accepted: 4 February 1981)

To study the correlation between heat treatment and changes in texture, carrots were used as model material. These were exposed to heat treatments of various duration and differing in their mode (cooking under atmospheric pressure, in water in the traditional way and at $5.3 \cdot 10^4$ Pa over-pressure in steam). The texture of individual samples was measured with the *Tensitest 500 Food* apparatus and in parallel subjected to sensory evaluation by ranking. The objective measurements were correlated to the sensorially established optimum. The maximum force ($F_2$) needed to crush the samples was established on the basis of the texturograms and the deformation moduli ($E_d$) were calculated. Both characteristics proved to be suitable for the description of texture found to be of good quality by sensory evaluation. The two values are as follows:

$F_2 = 3.9—5.6$ N $\qquad E_d = 5.0 \cdot 10^4—6.4 \cdot 10^4$ N m$^{-2}$.

During heat treatment, vegetables suffer various changes of which the change in texture is most easily perceptible and at the same time one of the most important ones. The texture of food is characterized by researchers by various mechanical characteristics in different grouping. Related theoretical problems and discussions are reviewed and evaluated by BIRÓ and co-workers (1977a).

KRAMER and SZCZESNIAK (1973) describe the five primary (hardness, cohesiveness, viscosity, springiness, adhesiveness) and three secondary (fracturability, chewiness, gumminess) characteristics the objective measurement of which permits the establishment of the so-called "texture profile". Since, however, the majority of foods is a complex disperse system and cannot be classified as solid or liquid material in the physical sense of the word, therefore the definitions or measurements as applied in rheology may not be applied without adaptation to foods. The mechanical principles as used in rheology, which form the basis of objective measurement of texture, *e.g.* viscosity and elasticity had to be interpreted afresh with the introduction of texture profile analysis. These interpretations do not correspond with the appropriate mechanical definitions. MOHSENIN (1970), ARNOLD and MOHSENIN (1971) and MOHSENIN and MITTAL (1977) suggest the introduction of the concept of "deformation modulus" instead of the "elasticity modulus" in the case of foods. While the "elasticity modulus" expresses the pressure–extension ratio in the elastic

domain of the material, to calculate extension in the case of the suggested "deformation modulus", the elastic and plastic deformation belonging to a selected point of the force-deformation curve as seen in Fig. 1, is used. The curve contains an inflection point characteristic of the complete deformation of the material.

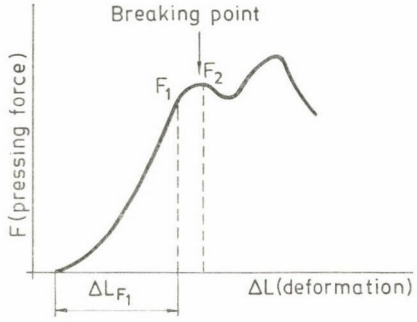


Fig. 1. Characteristic force-deformation diagram ($F_1$ is the force belonging to the 1st inflection point in the diagram). $\Delta L_{F_1}$ is the deformation belonging to force $F_1$. $F_2$ is the maximum force belonging to the point of rupture

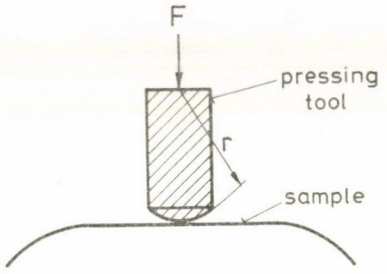


Fig. 2. Pressing tool applied in the determination of the deformation modulus of apple

These authors used for calculation the point of the curve which belongs to half of the complete deformation ($\Delta L_{F_1}$) extending to the first inflection point. To prove the applicability and correctness of their theory they carried out a rheological analysis of apple flesh by two different methods but both based on the theory of elasticity. In one of the methods the surface of the apple flesh was loaded with a rounded-off cylindrical press head and deformation modulus $E_d$ was determined from the shift caused by the force (Fig. 2).

To calculate the deformation modulus, the knowledge of the $\mu$ Poisson number, characteristic of the sample, is needed.

With the other method, the knowledge of the Poisson number is not required for the determination of the deformation modulus. In this case, cylindrical pieces were cut from the apple flesh and these were pressed between two plain surfaces (Fig. 3).

The deformation modulus was calculated in this case by the following formula

$$E_d = \frac{FL}{\Delta LA} \tag{1}$$

where

$E_d$ = deformation modulus
$F$ = half value of force $F_1$ belonging to the 1st inflection point of $F$-$D$ curve (Fig. 1)
$A$ = surface of the sample
$L$ = height of the sample
$\Delta L$ = deformation as caused by load $F$.

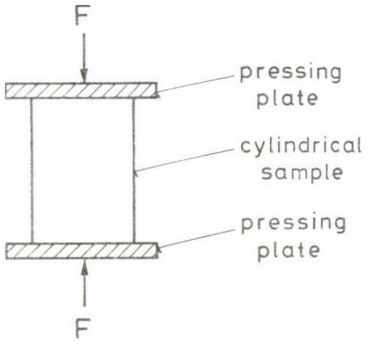


Fig. 3. The measuring outlay serving the determination of deformation modulus $E_d$

It was established that the value of the deformation modulus as calculated from data determined by the two methods did not differ significantly at the 95% probability level. The conclusion drawn from this investigation was that some of the rheological theories may be made use of in food research for practical purposes, although not all the conditions forming the basis of the theory are completely fulfilled.

A measuring design similar to the latter was applied by DRAKE and SELTSAM (1971) in the rheological analysis of raw carrots. GRÜNEWALD (1979) gives an account of his investigations into the texture of carrots raw, raw-frozen, precooked and frozen after precooking. ČELBA (1978) previously used the same method in his investigations.

MOHSENIN and MITTAL (1977) studied also the correctness of the statement of several authors according to which correlation exists between the deformation modulus of a solid food and the organoleptic sensation experienced upon chewing or biting.

They found that significant correlation exists only between the result of instrumental and sensory texture test if the load on the sample is similar in

both tests, that is the load is low or the sample is completely ruptured in both cases.

BOURNE (1977) studied sensory evaluation of the texture of solid foods and the pressure exerted upon the food in the mouth during chewing and the possible utilization of these sensations in the objective measurement of texture.

To measure the texture of foods several instruments were developed. The *Texturometer* and *Shear Press* instruments were constructed to measure the defined texture characteristics grouped according to texture profile analysis. A similar but further refined instrument was constructed in Hungary: the *Tensitest 500 Food*. This is described in detail by BIRÓ and co-workers (1977b).

## 1. Materials and methods

To develop an objective method for the establishment of the optimal heat treatment of vegetables by measurement of texture, carrots (variety: *Gonsenheim*) were used as a model. The variety studied was grown in Vecsés, it was used fresh and stored for a year, and cleaned prior to use. Part of the carrots was cooked in the traditional way under atmospheric pressure, covered with water, for 10, 15, 20, 25, 30, 35 and 40 min (1000 g of cleaned carrots in 1300 $cm^3$ water). The rest was steamed in a household pressure cooker *Kukta* under a pressure of 54 kPa for 5, 7, 10, 13 and 20 min, *resp.* (1 000 g cleaned carrots and 300 $cm^3$ water).

The texture of the samples was measured by the method as described by DRAKE and SELTSAM (1971), MOHSENIN and MITTAL (1977), GRÜNEWALD (1979) and ČELBA (1978). From the carrots of 25–30 mm diameter in the average, cooked at atmospheric pressure or steamed under pressure, cylindrical test samples of 20 mm height and 20 mm diameter were cut, possibly from the middle, taking into account the inhomogeneity of carrots. The test samples were compressed between two horizontal surfaces in *Tensitest 500 Food* apparatus as shown in Fig. 4.

The dashed line in the Figure marks the lower dead point, where the clearance between the two surfaces was reduced to 5 mm from the original 20 mm with a 15 mm stroke of the pressing tool.

The samples heat treated in two different ways for different length of time were subjected to sensory tests carried out by a panel of 5 members, applying evaluation by ranking. Two texturograms each were selected on the basis of these evaluations to represent the optimal doneness of samples cooked at atmospheric pressure or under pressure in steam, fresh and stored, respectively. Figure 5 shows the schematic outline of the texturograms of the samples optimal for consumption as obtained on the *Tensitest 500 Food* instrument.

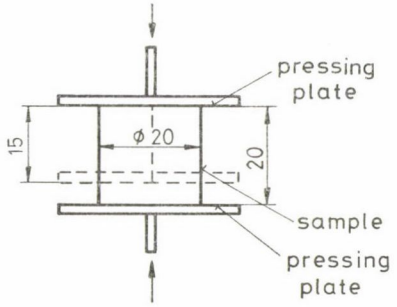


Fig. 4. Measuring outlay as applied in the rheological test of carrot samples

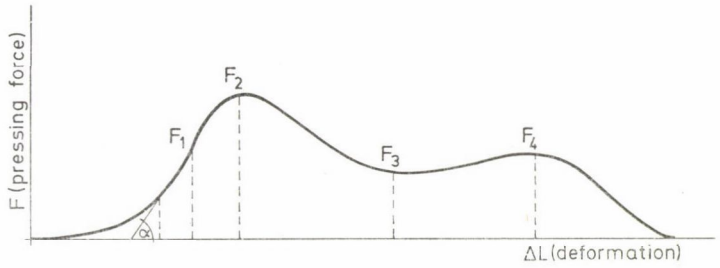


Fig. 5. Schematic diagram of force-deformation in carrot samples with the characteristic points

The symbols used in the diagram are as follows:

$F_1$ = force belonging to the inflection point of the initial ascending phase of the curve

$F_2$ = force required to the rupture of the material

$F_3$ = minimum of force subsequent to rupture of the material

$F_4$ = force needed to maximum compression to 15 mm

$\alpha$ = slope of the ascending linear phase of curve.

As can be seen in the Figure, first the test sample behaves as an elastic body, then upon the influence of force $F_2$ it ruptures. The further part of the curve belongs to the ruptured, rheologically new material. The curve has an inflection point at $F_1$ and here starts the change in texture. The initial phase is linear and ascends at angle $\alpha$. The shape of the curve corresponds to that published by MOHSENIN and MITTAL (1977) as seen in Fig. 1.

In evaluating the texturograms, the correlation between values $E_d$, tg $\alpha$, $F_2$ and $F_4$–$F_3$ and the sensory values was sought for. Deformation modulus $E_d$ was calculated by Equation 1. On the texturograms of carrots $F_{1/2}$ fell in every case outside of the ascending, approximately linear phase of the curve as seen in Fig. 5. Therefore, in calculating $E_d$, force $F_1$ and the deformation caused by it were taken into account. Force $F_1$ and deformation $\Delta L_1$ were determined from the texturograms.

## 2. Results

The texturograms of carrots showed substantial change in the course of cooking. The effect of different treatments is illustrated on randomly selected texturograms.

Figure 6 shows the texturograms of fresh carrot samples cooked in the traditional way at atmospheric pressure in water for 10, 15, 20, 25, 30, 35 and 40 min, respectively.

Figure 7 contains the texturograms of carrot samples stored for 1 year, cooked in the traditional way at atmospheric pressure in water for 15, 20, 25, 30, 35 and 40 min.

The force experienced at measuring the sample heated for 10 min, exceeded the force range of the measuring cell.

Figure 8 shows the texturograms of fresh carrots cooked in steam at over-pressure for 5, 7, 10, 13 and 20 min.

Figure 9 shows the texturograms of stored carrots cooked under pressure in steam for 7, 10, 13 and 20 min.

The texturogram of the sample cooked for 5 min is not included because the force elicited by its compression was outside the measuring range of the measuring cell and thus it could not be measured under the given conditions.

In Figs. 6–9 the texturograms of samples judged by the sensory panel as optimal texture were marked with asterisk (*). They seem to be very similar.

In the texturograms of the over-cooked samples, the characteristic points disappeared almost completely. This may be explained by the fact

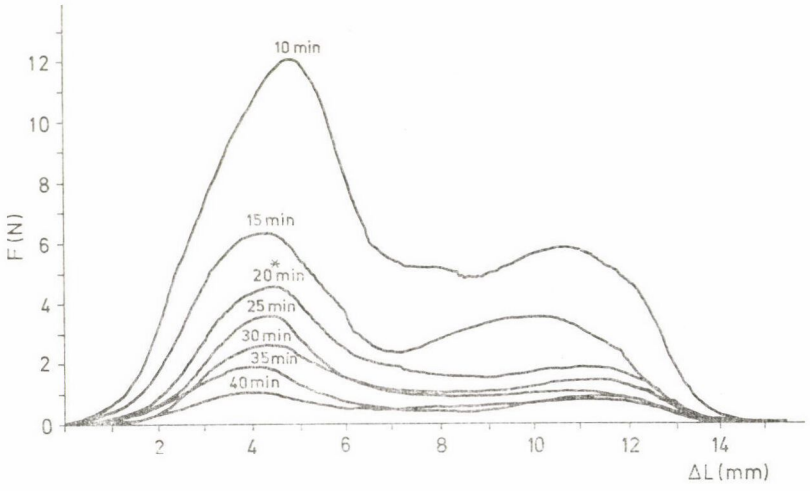


Fig. 6. Force-deformation diagrams of fresh carrot samples cooked at atmospheric pressure in water for different lengths of time. The diagram of samples judged by a sensory panel appropriate was marked by an asterisk (*)

that in the case of over-cooked samples the symptom of rupture did not occur since they had been pulpy.

In the following results are presented which may be correlated to sensory values.

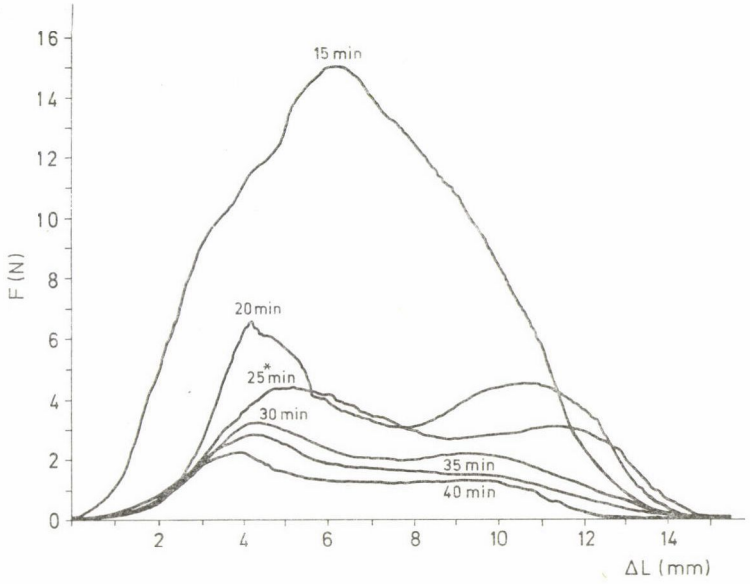


Fig. 7. Force-deformation diagrams of stored carrot samples cooked at atmospheric pressure in water for different lengths of time. The diagram of the sample judged appropriate by a sensory panel was marked by an asterisk (*)

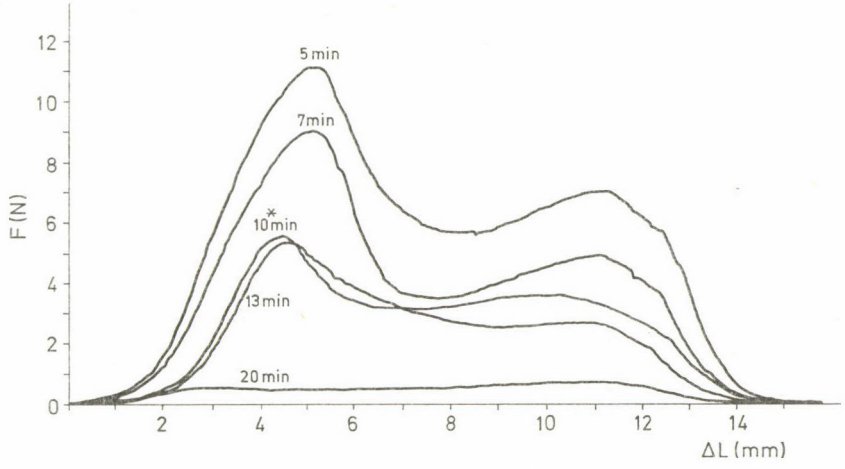


Fig. 8. Force-deformation diagram of fresh carrot samples steamed under pressure for different periods. The diagram of the sample judged appropriate by a sensory panel was marked by an asterisk (*)

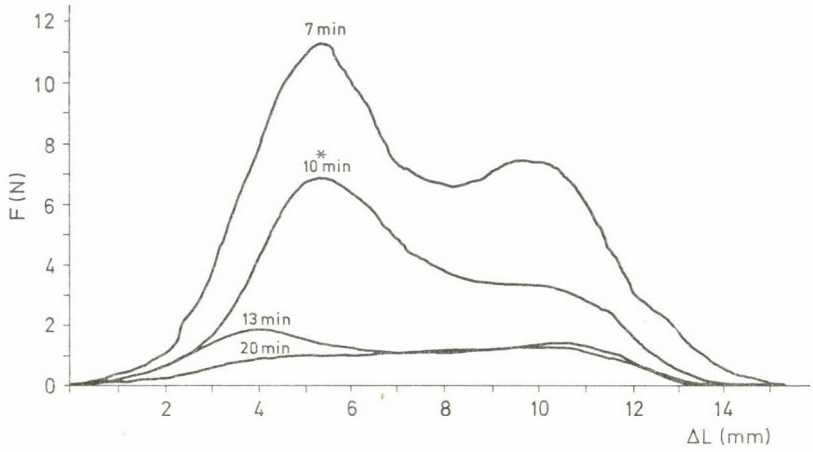


Fig. 9. Force-deformation diagrams of stored carrot samples steamed under pressure for different periods. The diagram of sample judged appropriate by a sensory panel was marked by an asterisk (*)

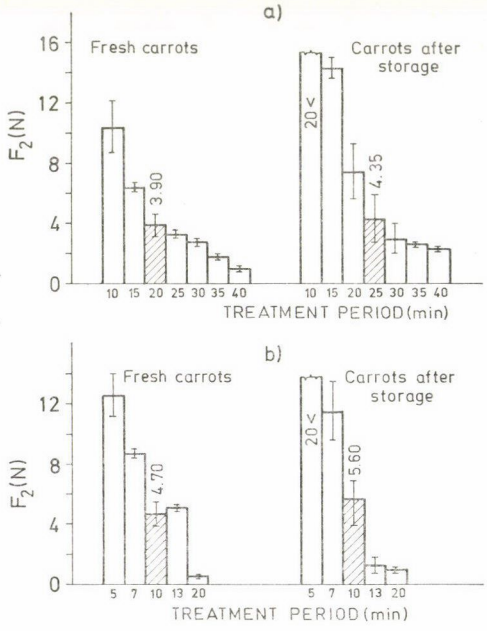


Fig. 10. $F_2$ values belonging to the rupture of fresh and stored carrot samples cooked at atmospheric pressure and under pressure for different periods. Columns of samples judged appropriate by the sensory panel are marked by shading

In Fig. 10 the $F_2$ forces needed to cause the rupture of fresh and stored carrots, cooked at atmospheric pressure for 10, 15, 20, 25, 30 and 40 min and of fresh and stored carrots cooked under pressure in steam for 5, 7, 10, 13 and 20 min, resp., are presented on column diagrams. The diagrams belonging to samples judged as of optimal texture by the panel were set off.

In Fig. 11 the deformation moduli ($E_d$) determined by Eq. 1 from the texturograms of fresh and stored carrot samples cooked in water in the traditional way for 10, 15, 20, 25, 30 and 40 min, and of fresh and stored carrots cooked under pressure in steam for 5, 7, 10, 13 and 20 min, resp., are illustrated on column diagrams. The diagrams belonging to samples judged as of optimal texture in sensory tests, were set off.

As can be seen in the Figures, values $F_2$ and $E_d$ decreased with increasing treatment periods in all four cases. In the samples sensorily appropriate (suitable for consumption), the value of $F_2$ varied between 3.9 and 5.6 N, while the value of $E_d$ between 50 and 60 kPa.

Values tg $\alpha$ and $F_4$–$F_3$ did not show a similarly unambiguous change as values $F_2$ and $E_d$. The change in $F_4$–$F_3$ could not be related to the sensory value. This proves also that the condition prior to rupture is sensed by the panel and values belonging to the condition after rupture are of no consequence in this study.

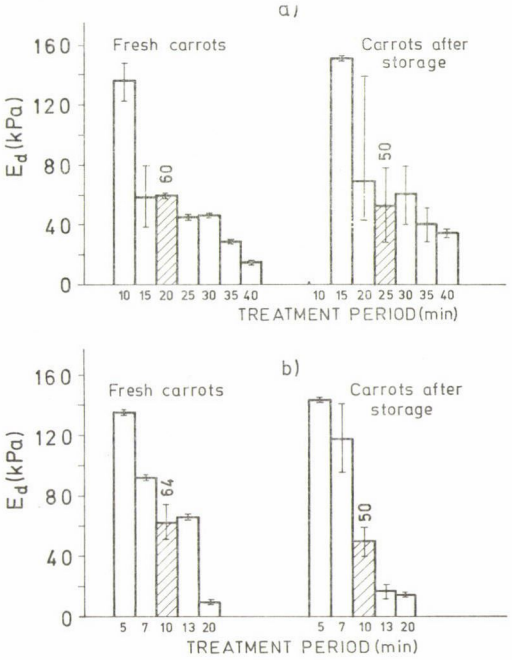


**Fig. 11.** Deformation moduli $E_d$ of fresh and stored carrot samples cooked at atmospheric pressure and over-pressure for different periods. Columns of samples considered appropriate by the sensory panel are marked by shading

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Addresses of the authors:

Ms. Magda HORVÁTH-MOSONYI — Department of Dietetic, Faculty of Advanced Paramedical Training at the Institute for Postgraduate Medical Education
H-1085 Budapest, Makarenko u. 24.
Hungary

Dr. Loránd HORVÁTH — Central Food Research Institute
H-1022 Budapest, Herman Ottó út 15.
Hungary

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# EXAMINATION OF THE VOLATILE CARBONYL FRACTION OF FRESH TOMATOES AND TOMATO PREPARATIONS

## I. THIN-LAYER-CHROMATOGRAPHIC SEPARATION

M. PETRÓ-TURZA and I. SZÁRFÖLDI-SZALMA

(Received: 6 January 1981; accepted: 3 February 1981)

A method has been developed for the extraction and thin-layer-chromatographic separation of volatile carbonyl compounds present in tomatoes and tomato preparations. Steam distillation, followed by precipitation with 2,4-dinitro phenylhydrazine (DNPH) was used for the extraction of the volatile carbonyl compounds. After washing and drying, the precipitate was dissolved in carbonyl-free ethyl acetate, followed by the thin-layer-chromatographic (TLC) separation of the carbonyl DNPH fraction on *Kieselgel* G, with developing solvent composed of 70 $cm^3$ diethyl amine, 29.5 $cm^3$ pyridine and 0.5 $cm^3$ water. Five carbonyl classes could be distinguished: aliphatic monoketones, aliphatic mono-aldehydes, unsaturated monocarbonyls, cyclic and aromatic carbonyls, and dicarbonyls.

The separations were repeated on 30 preparative layers (0.75 mm thick); the fractions were united and rechromatographed in another TLC system. Certain compounds could be tentatively identified on the basis of the $R_f$-value and spot colour of known carbonyl DNPH-s co-chromatographed by the samples. Thirty-nine carbonyl compounds could be detected, 25 of them identified by the above method in fresh tomatoes and tomato preparations. It was concluded that processing, *i.e.* production of tomato purée and powder changed the volatile carbonyl profile of fresh tomatoes significantly. While the monoaldehyde fraction (and mostly 2-hexenal) was dominant in fresh tomatoes, the cyclic carbonyls (and mostly furfural) and the dicarbonyls accounted for the major part of the total carbonyl content in tomato purée and powder. Also, the amount of unsaturated monocarbonyls decreased significantly in the latter samples. Obviously, the different volatile carbonyl profiles contribute to the observed differences in the flavour of fresh and processed tomatoes.

Tomato is known to contain over 400 volatile flavour components (VAN STRATEN, 1977; PETRÓ-TURZA *et al.*, 1977), the quality and quantitative ratios of these compounds determine the characteristic odour of tomato. The characteristic tomato flavour is due to these volatile compounds and the sugar–acid ratio the major factor of taste. According to previous experimental evidence, the quantity and quality of the volatile compounds of tomatoes are profoundly changed by industrial processing. The amount of the original, highly volatile compounds decreases considerably and new, volatile compounds are formed by heat decomposition of some of the non-volatile compounds (PETRÓ-TURZA & SZÁRFÖLDI-SZALMA, 1975). This results in the characteristic flavour of additive-free tomato preparations, which significantly differs from that of the original raw material.

If high quality raw material and mild processing conditions are used, the products have a pleasant flavour. However, these requirements are not

always fulfilled, so the quality of the tomato products is widely fluctuating. One of the basic requirements of uniformly high quality tomato products is the guarantee of the required flavour composition. However, a long and systematic research effort is required to identify the most influential flavour components and their optimum concentration ratios. Part of this work, the extraction, separation and identification of the volatile carbonyl compounds of fresh tomatoes and tomato preparations will be presented here.

## 1. Materials and methods

### *1.1. Materials used for the TLC separation of carbonyl compounds*

The volatile carbonyl compounds of tomato were separated and identified by TLC, as 2,4-dinitrophenyl-hydrazine (DNPH) derivatives. The model DNPH derivatives used for the development of the method and the identification of the components of the tomato samples were prepared from the following chemicals:

| | |
|---|---|
| formaldehyde | *Chemapol* (36–38% aqueous solution) |
| acetaldehyde | *Fluka*, puriss |
| propionaldehyde | *Fluka*, puriss |
| n-butanal | *Fluka*, puriss |
| i-butanal | *Fluka*, purum |
| n-pentanal | *Schuchardt* (95%) |
| i-pentanal | *Fluka*, purum |
| n-hexanal | *Fluka*, purum |
| n-heptanal | *Fluka*, puriss |
| n-octanal | *Schuchardt*, rein |
| acetone | *Reanal*, reagent grade |
| 2-butanone | *Reanal*, reagent grade |
| 2-pentanone | *Fluka*, purum |
| 2-hexanone | *Fluka*, puriss |
| 2-heptanone | *Schuchardt*, rein |
| 2-octanone | *Fluka*, purum |
| 6-methyl-5-heptene-2-one | *Fluka*, purum |
| 3-pentanone | *Schuchardt*, rein |
| furfural | *Fluka*, puriss |
| benzaldehyde | *Reanal*, reagent grade |
| acetophenone | *Reanal*, reagent grade |
| 2-propenal | *Fluka*, pract. |
| 2-butenal | *Fluka*, puriss |
| 2-hexenal | *Fluka*, pract. |

| | |
|---|---|
| glyoxal | *Fluka*, pract. |
| methylglyoxal | *Sigma*, grade II. |
| 2,3-butanedione | *Fluka*, puriss |
| 2,3-pentadione | *Fluka*, pract. |
| 2,4-pentadione | *Fluka*, puriss |

*1.2. Tomato samples used for the experiments*

Commercial fresh tomatoes and tomato purée were used. Fresh tomato (cultivar Kecskemét) was hand picked, washed, dried and processed. The tomato purée used was of the so-called *Golden Pheasant* brand produced by the HATVAN CANNING FACTORY, Hungary.

Since no tomato powder was available commercially, the samples tested were obtained from the KECSKEMÉT CANNING FACTORY, Hungary.

*1.3. Extraction of the volatile carbonyl compounds*

Steam distillation was used for the extraction of the volatile carbonyls from the fresh tomato samples and tomato preparations. Based on the investigations of KAZENIAC and HALL (1970) 3000 g fresh tomato were minced, homogenized and steam-distilled. The distillation product was collected into 150 $cm^3$ 2 *N* HCl solution containing 0.24% 2.4-dinitrophenyl-hydrazine. The receiver was cooled by ice-water. Since KAZENIAC and HALL (1970) concluded that the first 300 $cm^3$ fraction contained 85–90% of the volatile content of the samples, only this first fraction was collected.

Six-hundred and thirty g tomato purée and 196 g tomato powder were diluted with distilled water to 3 000 $cm^3$ and subjected to the same steam distillation process. Thus, all three materials tested were compared on identical dry material basis.

In order to ensure full precipitation the reaction mixture was allowed to stand overnight at ambient temperature then was kept at 50–60 °C for 15 minutes, finally cooled in ice-water. The precipitate was collected on a grade G4 glass filter. The filtrate was boiled for 20 minutes to precipitate the di- and oxi-carbonyl DNPHs. The solution was cooled to ambient temperature the precipitate was filtered on the same filter which contained the previous fraction of the DNPH precipitate.

The precipitate was washed first with 2 *N* HCl, then with distilled water. The precipitate was dried, then dissolved in carbonyl-free warm ethyl acetate. This stock solution was used for the TLC separations.

*1.4. TLC separation of the carbonyl DNPH samples*

The multistep TLC method proposed by KOZMA-KOVÁCS (1976) was tried for the separation and identification of the volatile carbonyl compounds extracted from the tomato samples.

*1.4.1. Pre-separation into classes of the carbonyl DNPH samples.* The first step of the TLC separation scheme is the separation into aliphatic monocarbonyl DNPH, aromatic monocarbonyl DNPH and dicarbonyl DNPH fractions of the original DNPH sample. The separation was first attempted on a zinc carbonate layer by a pyridine : water (99.5 : 0.5) solvent mixture as suggested by BEYER and KARGL (1972). However, sufficient separation as reported by KOZMA-KOVÁCS (1976) could not be achieved. The separation could not be improved either by the fractionation of zinc carbonate, different activation of the layers, or the changing of the ratio of the components of the solvent.

Therefore, a new TLC system had to be sought. A commercial adsorbent of standard composition and uniform quality, *Kieselgel* G had been selected. The composition of the mobile phase was varied until sufficient separation was obtained. Eventually, the separation shown in Fig. 1 could be obtained on *Kieselgel* G, activated for 30 min at 110 °C, with a pyridine : diethylamine (99 : 1) solvent mixture.

The retention order of the carbonyl classes was the same as on the zinc carbonate layer. The dicarbonyl DNPH fraction — a blue spot caused by the basic medium — is immediately above the start line, followed by the red spot of cyclic and aromatic monocarbonyl DNPH, and above it is the red-brown spot of the aliphatic monocarbonyl DNPH fraction. The separation is at least as good as on the zinc carbonate layer, and it has the additional advantage that the *Kieselgel* G layer is always available, so the results will be reproducible.

In the following step experiments were carried out to separate the monocarbonyl DNPH fraction into several subfractions (saturated aldehydes, saturated ketones and unsaturated monocarbonyls).

This separation was expected to save considerable time and effort in the course of the regularly tedious TLC separation scheme.

*Kieselgel* G activated for 30 min at 120 °C and diethylamine as solvent allowed for the separation of the enal DNPH fraction and at least partial separation of the aldehydes and ketones (Fig. 2).

In order to improve the separation, pyridine was mixed in various ratios with diethylamine used as the mobile phase. The results of these experiments are summarized in Fig. 3.

It can be seen that the separation of the aldehyde, ketone and enal DNPH fractions with pure diethylamine as mobile phase is insufficient for preparative purposes. This is in agreement with our former experimental findings.

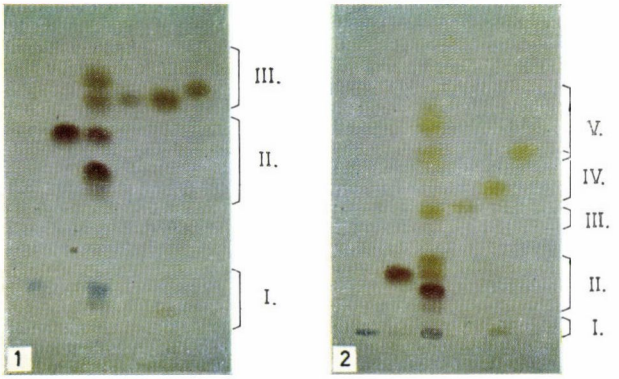


Fig. 1. TLC pre-separation of the mixture of model carbonyl-DNPHs into classes. Adsorbent: *Kieselgel* G (activated for 30 min at 110 °C); Solvent mixture: Pyridine-diethylamine (99 : 1); Samples from left to right: methyl-glyoxal; benzaldehyde; mixture of model carbonyls (see in Part 1.1); 2-hexenal; acetaldehyde; acetone. I: class of dicarbonyl compounds; II: class of cyclic and aromatic mono-carbonyl compounds; III: class of aliphatic monocarbonyl compounds

Fig. 2. TLC pre-separation of the mixture of model carbonyl-DNPHs into classes. Adsorbent: *Kieselgel* G (activated for 30 min at 120 °C); Solvent: Diethylamine; Samples from left to right: see in Fig. 1; I: class of dicarbonyl compounds; II: class of cyclic and aromatic mono-carbonyl compounds; III: class of unsaturated monocarbonyl compounds; IV: class of saturated monoaldehyde compounds; V: class of saturated mono-ketone compounds

The separation is better when the mobile phase is composed of 25 $cm^3$ pyridine and 75 $cm^3$ diethylamine. When the pyridine content of the mobile phase is higher than that, the separation deteriorates again. Thus, even though some pyridine in the eluent has improved the separation, it is not yet sufficient. Therefore, a number of polar solvents were tested as third component and their effects upon the separation were noted. Several alcohols, acetic acid and water were tried. The best results were obtained with water. The water content of the mobile phase significantly influenced the separation. Reproducible results could be obtained only when the water content of the eluent was strictly controlled. Therefore, both the chromatographic tank, the filter paper used to line the tank and the layer were carefully activated for 30 min at 110 °C.

The composition of the mobile phase yielding the best separation was 29.5 $cm^3$ pyridine, 70 $cm^3$ diethylamine and 0.5 $cm^3$ water.

The separation is shown in Fig. 4.

In this system the distance between the unsaturated monocarbonyl fraction and the aldehyde fraction is large, so their mutual contamination is highly unlikely.

The aldehyde fraction collected between the spots of acetaldehyde (the most retained aldehyde) and acetone (the most retained ketone) is probably

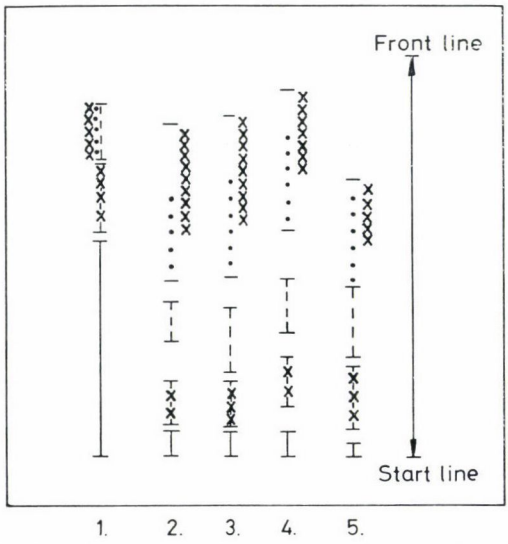


Fig. 3. Scheme of the TLC pre-separation of the mixture of model carbonyl DNPHs into classes on *Kieselgel* G layer using a mobile phase of pyridine and diethylamine in various ratios.
Composition of the mobile phase: 1. pyridine; 2. pyridine-diethylamine (75 : 25); 3. pyridine-diethylamine (50 : 50); 4. pyridine-diethylamine (25 : 75); 5. diethylamine × × × class of saturated monoketone compunds; . . . . . class of saturated monoaldehyde compounds; - - - - - - - class of unsaturated monocarbonyl compounds; - × - × - × - class of cyclic and aromatic carbonyl compounds; — — — class of dicarbonyl compounds

also free of ketones. Only the ketone fraction can be contaminated with $C_7$ and higher aldehydes. The separation of the ketone and aldehyde fraction could be further improved by using 20×40 cm layers. However, the use of such long layers was cumbersome, so it was discontinued, even though in this case only $C_8$ and higher aldehydes contaminated the ketone fraction. Probably, even better separation could be achieved on the recently introduced high performance thin-layers (HPTLC). However, such layers were not available for us at the time of the experiments.

Since the amount of the ketone fraction was low in the samples tested, no further attempt was made to improve the separation.

*1.4.2. Further separation of the carbonyl fractions obtained in the pre-separation step.* The raw carbonyl DNPH precipitate was pre-separated into several classes on 0.75 mm thick *Kieselgel* G preparative layers as described above. The spots were scraped off from 25–30 layers and united. The carbonyl DNPHs were eluted from the adsorbent with ethyl acetate, filtered and the filtrates were evaporated to dryness.

The saturated ketone and aldehyde DNPH fractions could be further separated on *Kieselguhr*, impregnated with *Carbowax* 400, using a c-hexane eluent saturated with *Carbowax* 400, as described by WALTHER (1967).

The unsaturated monocarbonyl DNPH fraction could be further separated on *Kieselguhr* G impregnated with 33.3% *Carbowax* 400, using a petroleum ether (bp 100–120 °C) eluent, as outlined by MEIJBOOM (1966).

*Kieselguhr* G, impregnated with polyethylene-glycol 400, and di-n-butyl-ether, as published by BADINGS and WASSINK (1963), were used for the separation of the components of the aromatic carbonyl DNPH fraction.

The dicarbonyl DNPH fraction was further studied on *Kieselgel* G activated for 20 min at 105 °C using an eluent composed of 97.5 parts toluene and 2.5 parts ethyl acetate.

## 2. Results and conclusions

The volatile carbonyl compounds of the samples were extracted by steam distillation and precipitated by DNPH as described in 1.3., then preseparated into classes as described in 1.4.1. The chromatogram obtained is shown in Fig. 5.

The differences between the volatile carbonyl profiles of fresh tomatoes, tomato purée and powder are apparent even in this chromatogram obtained at the preseparation step. While the unsaturated monocarbonyl fraction dominates in the fresh tomato sample, the amount of the aromatic and dicarbonyl fractions is much higher and that of the unsaturated carbonyl fraction is much lower in the purée and powder samples. The powder sample contained the least amount of carbonyls.

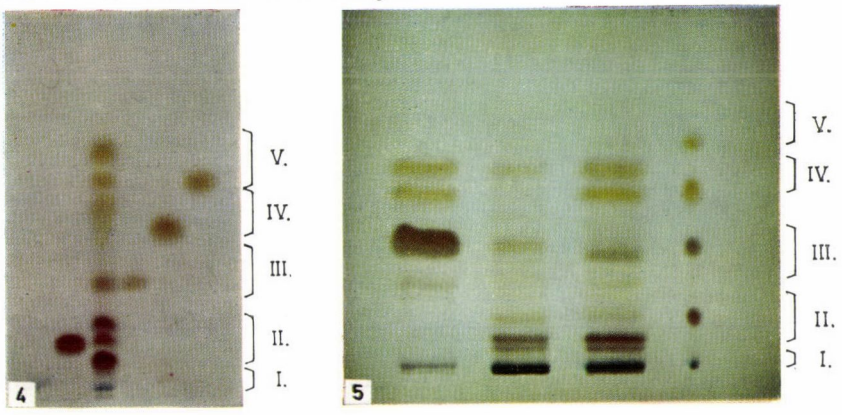


Fig. 4. TLC pre-separation of the mixture of model carbonyl DNPHs into classes. Adsorbent: *Kieselgel* G; Solvent mixture: 70 ml diethylamine + 29.5 ml pyridine + 0.5 ml water; Samples from left to right and numbers of classes see in Figs. 1 and 2

Fig. 5. TLC pre-separation into classes of the total carbonyl-DNPH fractions of the tomato samples.
Adsorbent, solvent mixture and numbers of classes see in Fig. 4; Samples from left to right: fresh tomatoes; tomato purée; tomato powder; model mixture: acetone, acetaldehyde, 2-hexenal, benzaldehyde, methylglyoxal

The repeated TLC separations of the carbonyl DNPH classes collected from the preparative layers resulted in the chromatograms shown in Figs. 6–10.

The rechromatographed fractions proved rather pure and contamination could be detected only occasionally.

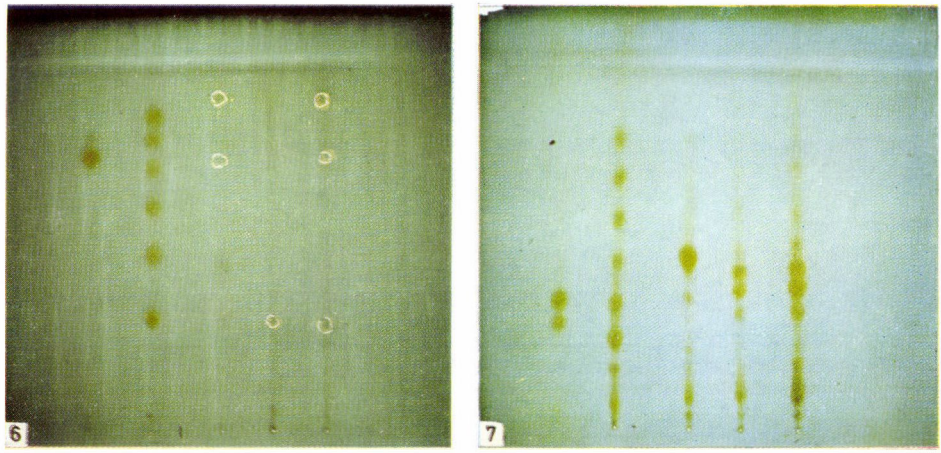


Fig. 6. TLC separation of the saturated monoketone DNPH fractions into homologues. Adsorbent: *Kieselguhr* (impregnated with *Carbowax* 400); Solvent: c-hexane saturated with *Carbowax* 400; Samples from left to right: 6-methyl-5-heptene-2-one; mixture of methyl-ketone homologues ($C_3$–$C_8$); fresh tomatoes; tomato purée; tomato powder

Fig. 7. TLC separation of the saturated monoaldehyde DNPH fractions into homologues. Adsorbent and solvent: see Fig. 6; Samples from left to right: mixture of i-butyr- and i-valeraldehydes; mixture of aliphatic aldehyde homologues ($C_1$–$C_8$); fresh tomatoes, tomato purée; tomato powder

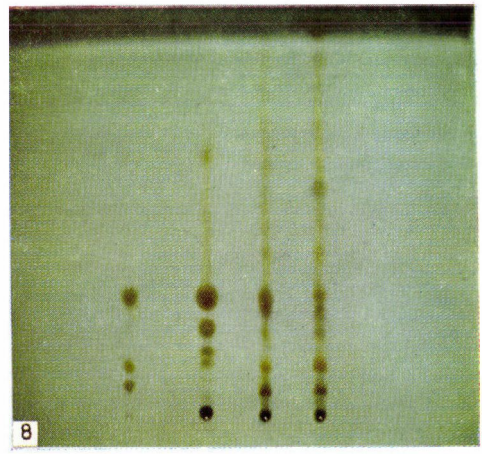


Fig. 8. TLC separation of the unsaturated monocarbonyl DNPH fractions into compounds. Adsorbent: *Kieselguhr* G impregnated with 33.3% *Carbowax* 400; Solvent: petroleum ether (bp. 100–120 °C); Samples from left to right: mixtures of 2-propenal, 2-butenal and 2-hexenal; fresh tomatoes; tomato purée; tomato powder

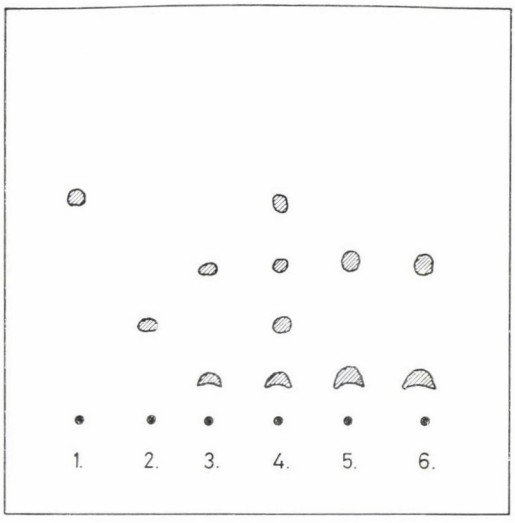


Fig. 9. TLC separation of cyclic and aromatic carbonyl DNPH fractions. Adsorbent: *Kieselguhr* G impregnated with polyethyleneglycol 400; Solvent: di-n-butylether; Samples from left to right: acetophenone; benzaldehyde; furfural; fresh tomatoes; tomato purée; tomato powder

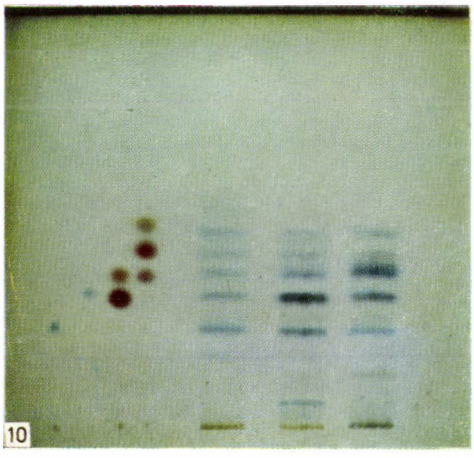


Fig. 10. TLC separation of the dicarbonyl DNPH fractions into compounds. Adsorbent: *Kieselgel* G activated for 20 min at 105 °C; Solvent mixture: toluene–ethyl-acetate (97.5 $cm^3$ : 2.5 $cm^3$); Samples from left to right: glyoxal; methylglyoxal; 2,3-butanedione*; 2,3-pentadione*; fresh tomatoes; tomato purée; tomato powder

* Red spot: only one carbonyl group reacted with DNPH; blue spot: (slightly above the red spots) both carbonyl groups reacted with DNPH.

The spots in these chromatograms could be identified by co-chromatographing known standards and comparing their $R_f$ values and colours. The components were thus tentatively identified and their estimated quantities are listed in Table 1.

These experiments were repeated in two successive years. It could be concluded that the major tendencies were identical, only the overall amount of the carbonyls and the number of the detectable minor components varied slightly. The amount of a number of selected carbonyl compounds also differed in the two successive years (*cf.* acetaldehyde; i-butyraldehyde; acetone; 6-methyl-5-heptene-2-one; 2-butenal; 2-pentenal; glyoxal; 2,3-butanedione). These differences are probably due to the slightly different raw material and the minor variations of the production technology.

Apart from the components tentatively identified, a couple of other spots appeared as well. These spots could not be correlated with any of the standards at our disposal. However, their colour indicated that they also belonged to the same carbonyl group. Their number and amount varied in the samples as follows. In the unsaturated monocarbonyl fraction:

| | |
|---|---|
| in the fresh tomato sample: | 1 minor spot |
| in the purée sample: | 1 medium large spot<br>3 minor spots<br>1 trace spot |
| in the powder sample: | 1 large spot<br>1 medium large spot<br>2 minor spots |

These spots can be attributed to branched 2-enals, other enals and dienals

In the dicarbonyl fraction:

| | |
|---|---|
| in the fresh tomato sample: | 4 minor spots<br>5 trace spots |
| in the purée sample: | 3 medium large spots<br>1 minor spot<br>4 trace spots |
| in the powder sample: | 1 large spot<br>1 medium large spot<br>1 minor spot<br>5 trace spots |

Table 1

*Volatile carbonyl compounds tentatively identified in fresh tomatoes, tomato purée and powder samples*

| Number | Name | Fresh tomatoes | | Tomato purée | | Tomato powder | |
|---|---|---|---|---|---|---|---|
| | | year | | year | | year | |
| | | 1978 | 1979 | 1978 | 1979 | 1978 | 1979 |
| | *Aliphatic monoaldehyde* | | | | | | |
| 1. | formaldehyde | S | S | Ø | S | Ø | S |
| 2. | acetaldehyde | L | M | M | M | S | L |
| 3. | propionaldehyde | M | S | Ø | S | S | S |
| 4. | i-butiraldehyde | T | S | T | M | T | M |
| 5. | i-valeraldehyde and/or n-valeraldehyde | M | S | S | M | S | M |
| 6. | capronaldehyde | LL | LL | S | S | S | S |
| 7. | n-heptanal | Ø | Ø | Ø | S | S | S |
| 8. | n-octanal | Ø | Ø | Ø | T | Ø | T |
| 9. | n-nonanal* | Ø | T | Ø | Ø | Ø | Ø |
| | *Aliphatic ketones* | | | | | | |
| 10. | acetone | T | T | T | M | T | M |
| 11. | 2-nonanone* | Ø | T | Ø | Ø | Ø | T |
| 12. | 6-methyl-5-heptene-2-one | L | T | T | Ø | M | T |
| | *Aliphatic enals* | | | | | | |
| 13. | 2-propenal | Ø | Ø | Ø | M | Ø | M |
| 14. | 2-butenal | Ø | S | Ø | M | Ø | M |
| 15. | 2-pentenal* | Ø | L | Ø | S | Ø | S |
| 16. | 2-hexenal | LLL | LLL | M | M | M | S |
| 17. | 2-nonenal* | Ø | S | Ø | S | Ø | S |
| 18. | 2-decenal* | Ø | Ø | Ø | S | Ø | Ø |
| | *Cyclic and aromatic carbonyls* | | | | | | |
| 19. | furfural | T | T | L | L | L | L |
| 20. | benzaldehyde | S | S | T | T | T | T |
| 21. | acetophenone | S | S | T | T | T | T |
| | *Dicarbonyls* | | | | | | |
| 22. | glyoxal | S | M | S | L | L | S |
| 23. | methylglyoxal | S | M | L | LL | L | L |
| 24. | 2,3-butanedione | L | M | L | S | LL | M |
| 25. | 2,3-pentadione | S | M | S | S | S | M |

Ø = non detectable
T = trace amount
M = medium amount
LL = very large amount
S = small amount
L = large amount
LLL = exceedingly large amount

* Compounds identified by their $R_f$ values only, as determined from the $R_f$ *vs.* carbon number relationship of the various homologous series.

Therefore, it can be concluded that the multistep TLC method suggested by KOZMA-KOVÁCS (1976) for the separation of the volatile carbonyl compounds could be considerably simplified by improving the efficiency of the pre-separation step. The new method has been successfully used for the examination of the processing-induced changes of the volatile carbonyl profiles. The quantities of the respective carbonyl compounds, and their effects upon the flavour of the tomato preparations will be dealt with in a forthcoming publication.

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Address of the authors:

Dr. Márta PETRÓ-TURZA
Ms. Ilona SZÁRFÖLDI-SZALMA

Central Food Research Institute
H-1022 Budapest, Herman Ottó út 15.
Hungary

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# INVESTIGATIONS ON THE MICRONUTRIENT SUPPLY OF THE DIET OF THE POPULATION IN NORTH KARELIA

S. Szalay, A. Murányi and A. Alasoini

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Mn, Cu and Zn contents of the food of North Karelian population are estimated, partly by analysis of cereal grains and cereal products, partly calculating the micronutrient intake utilizing the statistical data of the nutritional statistics of the population. Comparative analyses made on Hungarian food components are also presented. In North Karelia the daily average intake per person of Mn amounts to 3.29 mg, the Cu intake about to 1.07 mg and Zn intake to about 15.5 mg. The Mn and Cu intake is, according to international recommendations, either insufficient or at best marginal, the Zn intake is satisfactory. Cereals and from among them brown whole grain breads are the most important sources of Mn of which about 76% is supplied by cereals. The consumption of cereals decreased since the beginning of this century from about 600 g per day per person to about 250 g per day per person in this decade. The share of whole grain bread within this contingent decreased probably significantly as well and these factors affect undesiderably the Mn and Cu supply.

The incidence of cardiovascular diseases (CVD) is the highest in the world in Finland. Within Finland, North Karelia (NK) is still at some disadvantage in this respect (Keys, 1970; Bolander, 1971; Puska, 1973). The disease incidence and high mortality render it desirable to consider and investigate all possible factors which might contribute to this situation.

North Karelian soils are very unfavourable postglacial soils. They are formed from a few kinds of very old magmatic rocks. The last glaciation (Würmian) erased all older sedimentary soils. The micronutrient content of the bedrocks of this territory was investigated in a previous paper by Szalay and co-workers (1981). The analyses demonstrated that North Karelia is a Mn deficient geochemical province. Cu supply is not abundant there either, it is perhaps marginal, however Zn content of the bedrocks is about the same as the world average of continental soils. If it is preliminarily assumed as a working hypothesis that deficiency of some of the micronutrients might be causally correlated with some CVD cases, then Zn is out of suspicion. A possible role of Mn and Cu must be investigated.

These investigations are aimed at Mn, Cu and Zn, mainly because deficiencies of other micronutrients (*e.g.* Fe, I, F, Co) cause well known and diagnosed symptoms other than CVD in human beings. Although many biochemical roles of Cu, Mn and Zn are known, our knowledge about them is

very far from complete and if any of the micronutrients can be correlated with CVD they can be considered more seriously than others.

As known, many factors intervene in the food chain from bedrock to soil formation to plants, from plants to animals and to humans, which might modify the micronutrient supply of man. Further, significant part of the food is imported from other territories and countries and so it does not originate from North Karelian soil. Direct microanalytical investigations and calculations of the micronutrient content of the diet are reported in this paper which obviously circumvent the mentioned factors of the food chain.

## 1. Materials and methods

It is well known that the micronutrient content of plants is influenced by many factors. Micronutrient content of soil, particularly their mobility in the soils, weather, genetic factors of plant species, subspecies and individual plants, have all significant influences. Careful sampling methods are necessary to average these factors and obtain reliable, representative values.

The following sampling principles were followed throughout in this study. A single sample originated from many individual plants, amounted at first to about 100 g dry weight and it was crushed, ground and mixed. From this homogenized powder, about 5 g dry substance was weighed in for ashing. In the case of cereal grains, the grains originated of course from many plant individuals. North Karelian rye grains and bread samples were collected by the nutrition therapeutist of the HEALTH BOARD OF JOENSUU (NK), from different farm rye fields of various parts of NK and from bakeries and households, respectively. Hungarian cereal grain samples were obtained from storage silos or from commercial sources, so they represented mixtures from many fields and soils. They are compared here to the North Karelian samples.

The samples were dissolved after ashing at 500 °C for 4 h by a few drops of concentrated HCl. After evaporation they were taken up by HCl and diluted to a final defined volume of 0.1 *N* HCl. This solution was sprayed into the acetylene-air flame of an atomic absorption spectrometer. The following wavelengths were used:

| | |
|---|---|
| Mn: | 279.5 nm |
| Cu: | 324.8 nm |
| Zn: | 213.8 nm |

The accuracy of the atomic absorption spectrometer determination was better than 5%, optimally about 1%.

## 2. Results

### *2.1. Micronutrient content of cereals*

Great emphasis is attributed to cereals in this study because some analyses of food sources have already been published in the literature and among others carried out in this laboratory by MURÁNYI (1977), SZALAY and MURÁNYI (1982). These data demonstrate that cereals are the most abundant sources of some micronutrients, particularly of Mn.

Of the cereals, rye is grown in modest quantities in NK and it forms about 5–20% of the cereal supply of the population according to the amount of crop and season of the year. The addition of rye and wheat are imported from other parts of Finland, from the USSR and from other markets.

22 rye samples grown in 1979 in different parts of NK have been analyzed here in order to compare their micronutrient content with that of cereals (rye and wheat) grown in various soils of other countries (Hungary, USA). Table 1 demonstrates the results and mean values and standard errors of North Karelian rye samples.

The mean values of these data are compared in Table 2 with the mean values of the data of 12 rye samples collected from various places and soils of Hungary and of 29 winter wheat samples obtained from large blending storage silos of the HUNGARIAN STATE TRUST FOR CEREAL INDUSTRY. The samples originate and are blended from wheats grown in different parts and soils of Hungary in 1979. As known (UNDERWOOD, 1962), there is no significant difference between rye and wheat species concerning their micronutrient content. Rye and wheat can be compared here. Further data of USA summer (hard) wheats (CZERNIEJEWSKI *et al.*, 1964) are included in Table 2.

It is evident from Table 2 that rye samples grown in NK have an inferior Mn content, however, a normal Zn and Cu content. The Mn content is deficient by about 30%.

These analyses demonstrate that the Mn deficiency of North Karelian soils has a depressing effect on the Mn content of rye. It is probable that the same occurs with most plant products (potato, cattle-fodder *etc.*) grown in NK. It was not possible within this study to carry out detailed analyses of all of them.

However, the cereal grain supply of NK is only partly originating from the terrirory itself. The major part of rye and all wheat is imported from other territories of Finland — where the occurrence of CVD is similarly the highest on the world — and from the USSR, and other sources of the world market which are hardly possible to specify. It seemed to be indicated to analyze bread samples actually baked in NK without regard to the unknown origin of the grains or blend from which they are produced. Whole grain breads contain the largest concentration of micronutrients, so they have been

Table 1

*Micronutrient content of rye samples from different parts of North Karelia* (crops of 1979, grown on moraine soils)

| Origin of samples (Village, parish) | | Mn | Cu | Zn |
|---|---|---|---|---|
| | | ppm in dry weight | | |
| Kesälahti | Kesälahti | 20.3 | 4.44 | 25.2 |
| Salokyla | Kesälahti | 33.0 | 3.99 | 34.5 |
| Varmo | Kesälahti | 22.7 | 5.10 | 25.8 |
| Niinikumpu | Kitee | 26.5 | 5.34 | 27.7 |
| Rokkala | Kitee | 24.5 | 5.44 | 26.6 |
| Vaivo | Liperi | 40.2 | 5.25 | 31.7 |
| Leppälahti | Liperi | 28.3 | 4.95 | 35.0 |
| Kuorinka | Liperi | 35.4 | 4.93 | 36.4 |
| Käsämä | Liperi | 32.8 | 4.91 | 35.4 |
| Juuka | Juuka | 34.6 | 4.74 | 26.4 |
| Kannas | Juuka | 30.3 | 5.24 | 34.2 |
| Juuka kk. | Juuka | 42.3 | 5.53 | 28.1 |
| Kopravaara | Juuka | 39.5 | 5.51 | 36.0 |
| Paalasmaa | Juuka | 31.4 | 4.93 | 41.1 |
| Mannervaara | Ilomantsi | 26.1 | 4.23 | 23.4 |
| Tokrajärvi | Ilomantsi | 24.3 | 4.83 | 26.7 |
| Mutalahti | Ilomantsi | 28.5 | 4.05 | 26.6 |
| Karhunpää | Valtimo | 31.7 | 4.85 | 36.5 |
| Nurmeskylä | Nurmes | 21.8 | 3.73 | 40.2 |
| Jokikylä | Nurmes | 22,1 | 3,99 | 26,6 |
| Ruvaslahti | Polvijärvi | 30.2 | 4.60 | 33.5 |
| Selkäniemi | Polvijärvi | 29.3 | 4.31 | 35.5 |
| Mean value | | 29.8 | 4.77 | 31.5 |
| Standard error | | ±1.31 | ±0.11 | ±1.13 |

preferred in this analysis. Some bread samples made from husked and sieved rye flour are included which demonstrate that the flour depleted from its bran and germ content loses the largest part of its micronutrient content, particularly of its Mn content.

Data of North Karelian bread samples are presented in Table 3, related to the dry weight and wet (fresh) weight as well. Samples of the year 1979 from NK were obtained in a dried state. We measured the ratio of fresh consumption weight to the dry weight of rye breads and *Graham* breads in Hungary. The weight loss due to water evaporation was about 25%. The data

Table 2

*Comparison of micronutrient content of cereal grains of different origin*
(crops of 1979)
(Mean values ± standard errors)

| Number of samples | Sort of grain | Origin | Mn | Cu | Zn |
|---|---|---|---|---|---|
| | | | ppm in dry weight | | |
| 22 | rye (see Table 1) | North Karelia | 29.8 ±1.31 | 4.77 ±0.11 | 31.5 ±1.13 |
| 12 | rye | various places of Hungary | 45.3 ±0.86 | 3.60 ±0.05 | 27.3 ±0.20 |
| 29 | winter wheat | various places of Hungary | 35.3 ±1.22 | 4.15 ±0.15 | 27.4 ±1.52 |
| 10 | (hard) summer wheat | USA* | 46.0 ±2.54 | 5.30 ±0.34 | 35.0 ±1.58 |

* CZERNIEJEWSKI *et al.*, 1964

related to wet weight in Table 3 were calculated from the actually analyzed data of dry weight, corrected by this factor. Most of the grain used for cereal products was imported to NK, because of the unfavourable crop in 1979.

It has to be emphasized that the whole rye bread sorts of Table 3 represent in the statistical average of the population only a part of the total cereal consumption. These types of bread are the main micronutrient carriers, particularly Mn carriers. As no detailed data are available and obligatory official standardization of other bread sorts is not existing in NK, in assessing the micronutrient content of the more refined cereal products consumption our micronutrient data on Hungarian refined cereal products are utilized (see Table 4).

For comparison, the micronutrient contents of standard Hungarian bread sorts are demonstrated in Table 4 related to the dry weight and fresh weight. White bread is the overwhelmingly consumed standard wheat bread type of Hungary which is baked from a wheat flour of about 0.8% ash content. In addition analyses of white bread in the USA are included from literature (CZERNIEJEWSKI *et al.*, 1964). The inclusion of these data is necessary, because no wheat is grown in NK. All of it is imported. No significant errors are committed by using these data in calculating the micronutrient supply of the population of NK, because the daily wheat bread consumption is low, and because it can be assumed that Hungarian wheats approximate the world market average in micronutrient content. Our analytical data of Hungarian wheat flour products are used in calculating the micronutrient content of wheat flour products in Table 5.

Table 3

*Micronutrient content of whole grain breads from different parts of North Karelia* (baked in 1979)

| Ser. No. | Origin | Kind of bread | Mn | Cu | Zn | Mn | Cu | Zn |
|---|---|---|---|---|---|---|---|---|
| | | | ppm in dry weight | | | ppm in wet weight | | |
| 1 | Lieksa | whole rye (home made) | 23.9 | 4.77 | 26.0 | 17.8 | 3.54 | 19.4 |
| 2 | Ilomantsi | whole rye (home made) | 48.6 | 6.83 | 38.5 | 36.3 | 5.11 | 28.7 |
| 3 | Kitee | whole rye (home made) | 35.0 | 5.33 | 36.5 | 26.1 | 3.99 | 27.2 |
| 4 | Liperi | whole rye + 10% wheat + barley (home made) | 23.5 | 4.70 | 26.1 | 17.6 | 3.51 | 19.5 |
| Mean value 1–4 | | | 32.8 | 5.40 | 31.8 | 24.5 | 4.04 | 23.7 |
| Standard error± | | | 5.92 | 0.50 | 3.30 | 4.42 | 0.37 | 2.47 |
| 5 | Kontiolahti | 45% Finnish, 45% USSR whole rye + 10% wheat (home made) | 39.3 | 4.70 | 36.9 | 29.3 | 3.51 | 27.5 |
| 6 | Kontiolahti | | 33.1 | 7.94 | 36.3 | 24.7 | 5.93 | 27.0 |
| 7 | Lieksa | | 26.0 | 3.99 | 34.0 | 19.4 | 2.98 | 25.3 |
| Mean value 5–7 | | | 32.8 | 5.54 | 35.7 | 24.5 | 4.14 | 26.6 |
| Standard error± | | | 3.80 | 1.22 | 0.88 | 2.86 | 0.91 | 0.67 |
| 8 | Joensuu | Bakerie's whole rye | 22.4 | 3.60 | 33.8 | 16.7 | 2.69 | 25.2 |
| 9 | Joensuu | Bakerie's whole rye | 28.5 | 4.19 | 34.6 | 21.2 | 3.13 | 25.8 |
| 10 | Joensuu | Bakerie's whole rye | 26.1 | 3.89 | 26.2 | 19.5 | 2.91 | 19.6 |
| 11 | Ilomantsi | Bakerie's whole rye | 26.8 | 3.89 | 26.2 | 19.9 | 2.91 | 19.6 |
| 12 | Liperi | Bakerie's whole rye | 25.5 | 3.50 | 24.1 | 19.0 | 2.61 | 18.0 |
| 13 | Lieksa | Bakerie's whole rye | 27.5 | 3.84 | 34.0 | 20.5 | 2.87 | 25.4 |
| Mean value 8–13 | | | 26.1 | 3.82 | 29.8 | 19.5 | 2.85 | 22.3 |
| Standard error± | | | 0.90 | 0.10 | 1.96 | 0.64 | 0.08 | 1.46 |
| 14 | Joensuu | Bakerie's husked rye | 13.5 | 2.42 | 15.9 | 10.0 | 1.83 | 11.8 |
| 15 | Joensuu | Bakerie's husked rye | 14.9 | 2.70 | 21.0 | 11.1 | 2.01 | 15.6 |
| 16 | Joensuu | Bakerie's husked rye | 21.6 | 3.00 | 23.9 | 16.2 | 2.24 | 17.8 |
| 17 | Lieksa | Bakerie's husked rye | 15.4 | 2.50 | 16.0 | 11.5 | 1.87 | 11.9 |
| 18 | Ilomantsi | Bakerie's husked rye | 24.4 | 3.25 | 23.5 | 18.2 | 2.42 | 11.7 |
| 19 | Liperi | Bakerie's husked rye | 19.8 | 2.75 | 15.1 | 14.8 | 2.05 | 11.3 |
| Mean value 14–19 | | | 18.3 | 2.77 | 19.2 | 13.6 | 2.07 | 14.3 |
| Standard error± | | | 1.76 | 0.13 | 1.65 | 1.33 | 0.09 | 1.23 |
| 20 | Joensuu | mainly whole grain wheat (Graham) | 21.6 | 2.83 | 23.0 | 16.1 | 2.13 | 17.2 |

Table 4

*Micronutrient content of standard Hungarian bread sorts*

| No. of samples | Sort of bread | Origin | Mn | Cu | Zn | Mn | Cu | Zn |
|---|---|---|---|---|---|---|---|---|
| | | | mg per kg dry weight (mean value) | | | mg per kg wet weight (mean value) | | |
| 6 | Graham bread | Hungary | 22.50 | 2.55 | 13.60 | 16.20 | 1.84 | 9.80 |
| 6 | White bread | Hungary | 7.61 | 0.92 | 9.56 | 5.15 | 0.62 | 6.48 |
| 6 | 30% rye + 70% wheat bread | Hungary | 7.10 | 2.30 | 17.90 | 4.70 | 1.50 | 11.90 |
| 6 | Rye bread (70% rye + 30% wheat) | Hungary | 6.80 | 2.70 | 18.40 | 4.50 | 1.80 | 12.20 |
| 6 | Fine bakery products (roll of bread) | Hungary | 4.03 | 1.00 | 5.78 | 2.97 | 0.74 | 4.27 |
| 3 | Fine bakery products (white wheat loaf) | Hungary | 2.80 | 0.90 | 3.60 | 1.90 | 0.60 | 2.40 |
| 10 | White bread | USA* | 5.90 | 2.30 | 9.70 | | | |

* CZERNIEJEWSKI *et al.*, 1964

Table 5

*Information about the average consumption of cereals by the population of North Karelia* (related to fresh, wet weight: *a–e;* to dry weight: *f*)

*a, b, c* = direct analysis of North Karelian bread samples, mean values
*d, e, f* = calculated from the results of the analysis of Hungarian samples

| | Sort of bread | Daily consumption per person (g) | Mn | | Cu | | Zn | |
|---|---|---|---|---|---|---|---|---|
| | | | ppm | mg per day per person | ppm | mg per day per person | ppm | mg per day per person |
| *a* | Whole rye bread | 60 | 22.2 | 1.33 | 3.52 | 0.21 | 23.7 | 1.42 |
| *b* | Graham (whole grain wheat) bread | 15 | 16.1 | 0.24 | 2.13 | 0.032 | 17.2 | 0.26 |
| *c* | Husked rye bread | 40 | 13.6 | 0.54 | 2.07 | 0.083 | 14.3 | 0.57 |
| *d* | Yeast (wheat) bread | 15 | 5.15 | 0.077 | 0.62 | 0.009 | 6.48 | 0.097 |
| *e* | White wheat bread | 5 | 1.90 | 0.010 | 0.60 | 0.003 | 2.40 | 0.012 |
| *f* | Other white flour prod. (macaroni, dumplings, cakes, *etc.*) per dry weight | 115 | 2.64 | 0.30 | 1.20 | 0.14 | 4.20 | 0.48 |
| Total cereals per person per day<br>Total intake | | 250 | | 2.50 | | 0.48 | | 2.84 |

Very detailed investigations of the Mn, Cu and Zn content of various cereal products in Hungary were carried out in this laboratory (SZALAY and MURÁNYI, to be published). Because of centralized national economy and large scale state-owned cereal industry, inclusive silos, mills and bakeries, very reliable averaged samples and data on the composition of obligatory standardized sorts of breads and flours, refined flour products *etc.* are available. The study demonstrates that in the course of milling and refining procedure the micronutrient content of the flour mill cereal products decreases by the increasing quality (whiteness) of the flour. In fact, as known already (CZERNIEJEWSKI *et al.*, 1964), the mineral element content of cereals and the micronutrient content as well is strongly concentrated in the husk and germ, and in the course of milling and refining it remains largely in the bran and germ. Some considerations are necessary in comparing commercial cereal products of various countries particularly when composition and quality are not officially and obligatorily standardized. However, the ash content of the flour demonstrates the degree of refinement. Whole grain wheat has an ash content of about 1.8–2.0% and the whitest commercial sorts of wheat flour about 0.50–0.55%. Another kind of standardization is the extraction yield of flour. 78% extraction yield means that from 100 kg wheat 78 kg flour is obtained. 72% extraction flour is white flour and called "patent flour" or "refined white wheat flour" in the USA. (Determination of the ash content of bread is misleading because significant quantity of salt, minerals and yeast are added to the dough before baking.)

It is an important fact that the Mn content decreases very much with the decrease of ash content of the flour, while the Cu and Zn content are less affected. If the dietary habits of the population change from whole grain wheat bread or whole grain rye bread gradually to white bread sorts then the Mn supply decreases very significantly, but the Cu and Zn supply definitely less so.

Table 5 demonstrates in the first column the total cereal consumption of the North Karelian population specified for different cereal products estimated by the nutrition therapeutist of the HEALTH BOARD OF JOENSUU (NK) enquiring a well chosen representative part of the population in 1979. The data of bread sorts are related as usually to the fresh weight, however data of other white flour products to the dry weight as commercially sold. Logically, the data in the columns of micronutrients contain the concentration in ppm, or in mg per day per person, in case of bread sorts related to fresh weight and for other white flour products related to dry weight. In summarizing these columns we obtain the average micronutrient intake of the population from cereals in mg per day per person.

In Table 5 the first three (*a*, *b*, *c*) bread sorts were obtained from NK and the results are direct analyses of the samples but related to the fresh consumption weight. Sort *a* corresponds to the mean value of samples Ser.

Nos. 1–13 of Table 3, sort *b* to sample Ser. No. 20 of Table 3, *c* samples Ser. Nos. 14–19 of Table 3. Concerning *d*, *e*, *f* — the micronutrient content is calculated from analyses of comparable Hungarian products, *d* is approximated with the white bread of Table 4, *e* is approximated with white wheat loaf of Table 4, and *f* is approximated with the micronutrient content of refined white flour of Hungary of which the ash content is about 0.50–0.55%. It is emphasized that the final results in the total micronutrient consumption are not sensitive for this somewhat arbitrary comparison. By identifying the named North Karelian sorts with other Hungarian sorts the influence in the calculated Mn consumption is less than 0.1 mg Mn per day per person.

In adding the micronutrient contents of cereal products of Table 5 it is clear that the average micronutrient supply of the North Karelian population originating from cereals, is about 2.50 mg Mn per day per person, 0.48 mg Cu per day per person and 2.84 mg Zn per day per person. From the daily 2.50 mg Mn 2.11 mg is determined from direct analysis of North Karelian bread samples, 0.39 mg is estimated by using data of comparable Hungarian wheat products.

It will be demonstrated in the following that cereals are the main sources of Mn, as about 76% of the total Mn supply comes from them with 45% of Cu and 18% of Zn. Other food sources supply the rest.

### *2.2. Micronutrient content of other food sources*

Problems were encountered in obtaining sufficiently informative statistical data about the consumption of food products in NK. They have been collected from the general nutritional point of view and not from the point of view of micronutrient supply. The data from literature are partly somewhat incoherent, partly refer to different years. They refer partly to whole Finland and partly are specified for the single regions, among them NK. Fortunately, as it will be seen, cereals contain the largest part of the Mn and Cu micronutrient supply and so their influence on the total micronutrient intake is rather large. The contribution of other food sources is modest and so the micronutrient content of the total consumption is not very sensitive to the other sources. This fact enables the estimate of the mean value of micronutrient supply of the population with a considerable reliability.

Concerning the nutritional statistical data the following sources were utilized: HASUNEN and co-workers (1976) treated in great detail the food consumption in government districts (incl. NK) and within them 13 regions of Finland between the years 1969–1972. This study was carried out by a mobile autoclinic group survey enquiring a representatively chosen part of the population, organized by the INSTITUTION OF SOCIAL INSURANCE INVESTIGATION, Helsinki. Information about cereals is restricted to rye, wheat and other grain

consumption, without specified information about various refined cereal products. Male and female inhabitants were interrogated and data collected separately. From this statistical survey we utilized the data concerning the population of NK. Mean values of male and female inhabitants were calculated. However if we add the cereals (wheat + rye + other corns) it seems that the total consumption amounts to 360 g per day per person as a mean value for males and females for 1969–1972, in NK. This is in contradiction with other sources from 1977 (INFORMATION CENTRE OF AGRICULTURE, Maatalousalan Tiedetuskeskus) concerning whole Finland, and our own direct interrogation of the population of NK in 1979, resulting in about 250 g per day per person total cereal products. We assume that in the work of HASUNEN and co-workers (1976) the cereal consumption is represented in total consumed grain weight equivalent, the data of our inquiry and its results in Table 5 on the other hand are referred to the direct weight of final products. No sugar and fat consumption is specified in the study of HASUNEN and co-workers (1976) but it is probably included into the line of "other food stuffs". In the source of INFORMATION CENTRE OF AGRICULTURE a sugar consumption per day per person of about 101 g is mentioned. Sugar is very significant from the point of view of calories, as it supplies about 12% of them, but sugar and fats do not contain any micronutrients at all.

In Table 6 the consumed amounts of the most important sources of the diet of NK in 1969–1972 are represented, after HASUNEN and co-workers (1976) with the exception of data concerning cereals which are represented summarized according to our detailed nutritional survey and analyses, demonstrated already in Table 5.

The amounts of the products are given in fresh weight as consumed by the population. Micronutrient intakes are represented in the columns in mg per day per person.

Data concerning other kinds of food are estimated from our analytical data on samples grown in Hungary, using the NK data for the consumption. This is probably an overestimate, because of the Mn deficiency of NK soils. The juicy and fleshy part of a number of fruits was determined by MURÁNYI (1977) without seeds, it is between modest limits of min. 0.3 ppm Mn (lemon, oranges) up to maximum 1.0 ppm Mn (plums, bananas), and a number of others (apples, pears, peaches, apricots, cherries, grapes) is between 0.5–0.9 ppm Mn. We utilized the mean value of 10 kinds of fruits inclusively those above, the result being 0.67 ppm Mn. This mean value is utilized in Table 6, for the daily Mn intake data. We arrived by the same consideration at the Cu and Zn daily intake values in Table 6 for "fruits and berry fruits".

The contribution of berry fruits to the supply of Mn and Cu might be somewhat more significant. A number of Hungarian berry fruits were analyzed earlier in this laboratory, however the whole fruit has been ashed, including

Table 6

*Estimate of average daily micronutrient intake of the population of North Karelia in 1969–1972*

| Kind of food | Consumption (g per day per person) | Mn | Cu | Zn |
|---|---|---|---|---|
| | | mg per day per person | | |
| Cereals* | 250 | 2.50 | 0.48 | 2.84 |
| Potato | 184 | 0.16 | 0.13 | 0.50 |
| Meats | | | | |
| Beef and others | 63.7 | 0.025 | 0.035 | 3.79 |
| Ham, pork | 28 | 0.006 | 0.024 | 1.11 |
| Sausage | 57 | 0.013 | 0.050 | 2.27 |
| Blood and entrails | 6.33 | 0.006 | 0.0016 | 0.11 |
| Fish | 31.3 | 0.018 | 0.028 | 0.22 |
| Milk | 352.2 | 0.011 | 0.014 | 1.16 |
| Other (liquid) milk products | 521 | 0.016 | 0.021 | 1.72 |
| Butter | 42.8 | 0.0009 | 0.004 | 0.073 |
| Cheese | 12.2 | 0.002 | 0.004 | 0.45 |
| Margarin and oil | 3.5 | 0.00004 | 0.0001 | 0.0003 |
| Eggs | 31 | 0.009 | 0.003 | 0.361 |
| Fruits, berry fruits | 175.8 | 0.12 | 0.030 | 0.091 |
| Edible roots | 43.2 | 0.128 | 0.061 | 0.140 |
| Other vegetables | 69.8 | 0.156 | 0.111 | 0.395 |
| Legumes, nuts | 7.5 | 0.117 | 0.060 | 0.249 |
| Other food stuffs | 195 | | | |

* Taken from Table 5
Other consumption data from HASUNEN and co-workers (1976)

the seeds (MURÁNYI, 1977). As the seeds are indigestible and in all plants the seeds contain the highest concentration of micronutrients, these values are too high. A value of about 3.0 ppm is the content of Mn, 0.75 ppm of Cu and 2.3 ppm of Zn, as an average of three berry fruits (raspberry, strawberry, and currant), which are consumed in Hungary and in Finland as well.

No data were available about tea consumption in NK. Tea has a considerable Mn content of 0.29 mg Mn per cup when the brew is prepared from 1.5 g tea leaves per cup. We analyzed 14 tea sorts unpublished yet. The result was about 0.29 mg Mn per cup $\pm$ 0.025. Extreme values were 0.12–0.50 mg Mn per cup. The extremely high milk consumption in Finland and NK of about 870 g per day per person makes it probable that tea consumption is not significant.

All possible errors introduced by these estimates into the final result are not significant in comparison with the scattering of the statistical data of the consumption of the population. The standard deviation of the data of the

statistics in the material collected by the INSTITUTE OF SOCIAL INSURANCE INVESTIGATION OF FINLAND (HASUNEN *et al.*, 1976) demonstrate about almost 100% around the national average in various food sources, the smallest s.d. is ±57% from a mean value of 43 g butter per person per day. The dietary habits of the individuals deviate very much from the national average and so the mean values result from very scattered individual data. This study was aimed at gaining some preliminary information about the average micronutrient supply of the population, however the supply of any individual can differ from it very much according to his dietary habits.

## 3. Conclusions

It was stated in the foregoing paper (SZALAY *et al.*, 1981) that, from a geological point of view, NK is a manganese deficient geochemical province. Further, about 1/3 of the arable soils of NK consists of peat, and plants grown on peat are deficient in Mn and Cu (SZALAY *et al.*, 1975).

The population of this same region is suffering statistically the highest incidence of CVD casualties in the world (KEYS, 1970; BOLANDER, 1971; PUSKA, 1973). However, this correlation might not be a causal one because the number of CVD casualties increased in the course of the recent decades and the geochemistry of this territory is the same since millenia. Dietary habits and living circumstances have probably the most important role but the geochemical deficiency of Mn in the territory might be a contributing factor in the general supply of it.

By studying the nutrition in this work it is an analytically established fact that the average Mn content of a great number of rye samples grown in NK is about 30% less than that of rye and wheat in other countries (Table 2). The Cu and Zn contents are about normal. The influence of this Mn deficiency is not evident in the presently investigated bread sorts of NK. As mentioned before, the larger part of the cereal grain supply is imported depending upon the season and home grown crop of the year, and in the year of our investigations (1979) crop yields in NK were very low.

As Table 5 demonstrates, the total cereal consumption amounts to about 250 g per day per person of which about 100 g per day per person is rye bread. The consumption of whole grain bread sorts secures actually the largest part of Mn supply of the diet because the Mn content of other food sources including white bread and refined cereals is much smaller. In the course of the developing living standard, the total consumption of bread decreased in NK and Finland similarly to other countries. Data for whole Finland demonstrate that the whole mean consumption of cereals amounted in 1910 almost to 600 g per day per person and it decreased to 250 g per day

per person up to 1977. It can be assumed further that within this consumption the proportion of white refined cereal products to whole grain bread sorts was lower at the beginning of this century. The influence of this change of dietary habits upon the decrease of the Mn and Cu supply is very significant because refined cereals contain only a small part of them from the whole grains (SZALAY and MURÁNYI, to be published). The situation in dietary habits is in this respect more or less similar in most of the developed countries.

A great number of food sources dominating the nutrition of developed countries is not rich in Mn and Cu. Fats, oils, sugars, have no micronutrient content at all. Further the Mn and Cu contents of dairy products are almost negligible, in meats and fish Zn is abundant, however, Mn and Cu is very sparse with the exception of entrails, particularly liver which is an ample source of Mn, Cu, and Zn. Alcoholic beverages and industrial soft drinks do not contain micronutrients.

Cereals are the most important and reliable sources of micronutrients, particularly of Mn, however often all cereals are summarized in the nutritional statistics under this collective term. The differences among the micronutrient content of different cereal products are large because Mn, Cu and Zn are contained mainly in the husk and germ. The Mn content of whole grain bread sorts and brown breads is very high but that of refined white flours is much lower, most of the Mn being lost in the bran and germ. These facts must be considered from the point of view of nutritional studies on the Cu and Mn micronutrient supply. Data about the consumption of cereals should be very specified for the various types of cereal products, however the consumption of fats, oils, sugars, and dairy products is almost unimportant from the point of view of Mn and Cu supply.

Green, leafy vegetables contain significant concentrations of micronutrients, however, the consumed quantity is small and very different among persons and populations. No specification is made in the NK nutritional data about green leafy vegetables. Their annual average consumption is probably low due to the climate of the territory.

Cereals have the main role (about 50%) also in the supply of copper, however, many other food components (potato, meats) contribute significantly to it. In the supply with Zn, meats and cereals dominate and dairy products, eggs, *etc.* contribute rather significantly. The present contribution of cereals to Zn amounts to 18%.

Summarizing the results of this analytical study, it is established with certainity that the changing alimentary habits since the beginning of this century influenced the Mn and Cu supply to a very great extent. By the decrease of the consumption of cereals and particularly within the cereals the brown whole grain breads, the Mn supply decreased to a small part of that at the beginning of this century, when it was at least 10–15 mg per day per person

or more, when estimated from 600 g per day per person total cereal consumption. This loss is not compensated by the increasing consumption of sugar, fats, oils, meats, and milk products, *etc.*

International dietary recommendations concerning essential Mn requirement are somewhat vague, contradictory. They are treated in detail by UNDERWOOD (1971). The minimum requirement value is not known and human diseases caused by Mn deficiency are not recognized yet. Estimates concerning the supply of the population of NK arrived at in our paper in Table 6 are definitely lower, about 3.29 mg per day per person than earlier estimations in Finland and in NK which with a daily intake of 6–8 mg Mn per person per day, are too high (HASUNEN *et al.*, 1976). It seems that in statistical average the population of NK is undersupplied with Mn or at best marginally supplied. Persons deviating significantly from the mean values of the statistics by particularly low consumption of whole grain bread sorts are definitely undersupplied. Because affluence generally decreases the consumption of cereals and whole grain breads there seems to be a strong tendency of decrease of Mn intake in affluent developed nations, if the Mn intake is not supplemented by other food sources.

The Cu supply of 1.07 mg per day per person seems to be too low or marginal as well. Requirement estimates in literature amount to about 2 mg Cu per person per day (CARTWRIGHT, 1950).

Zn supply seems to be sufficient: at 15.50 mg per person per day it corresponds to the established recommendations. The Zn supply comes from various sources, in addition to cereals, mainly from dairy products and meats, so the decrease of whole grain bread consumption is compensated by the other sources.

In summary, it is emphasized that there is a correlation, as a function of time, between the decreased Mn and Cu supply of the population in the course of this century and the increase of the incidence of cardiovascular diseases in the same period.

It must be pointed out, however, that although this correlation is definitely existing, it is not a direct evidence of a causal correlation. Cardiovascular diseases are regarded as multiaetiological and the most significant factors causing them are probably genetic factors of the constitution of the individual. This weakness is activated by environmental influences. The decreased Mn and Cu supply is pointed out in this study. There seems to be a serious possibility that among other environmental risk factors Mn and/or Cu deficiency might contribute to a part of cardiovascular diseases.

If a direct causal correlation could be proved, the Mn and Cu supply should be enhanced to the level of the beginning of this century. This is easily possible without giving up the present dietary habits, some advantages of which (copious supply of animal proteins, easy digestibility, *etc.*) are obvious.

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Addresses of the authors:

Dr. Sándor SZALAY
Dr. Annamária MURÁNYI
Institute for Nuclear Research of the Hungarian Academy of Sciences
H-4001 Debrecen, Bem tér 18/c.
Hungary

Dr. Antti ALASOINI
Health Board of Joensuu
Kauppakatu 27, 80 100 Joensuu 10.
Finland

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# EXAMINATION OF THE VOLATILE CARBONYL FRACTION OF FRESH TOMATOES AND TOMATO PREPARATIONS

## II. HIGH PERFORMANCE LIQUID-CHROMATOGRAPHIC SEPARATIONS

Z. VARGA-PUCHONY, GY. VIGH, J. HLAVAY, M. PETRÓ-TURZA and I. SZÁRFÖLDI-SZALMA

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Carbonyl compounds profoundly influence both the taste and the odour of food. In order to improve the flavour of the processed food products, the carbonyl containing compounds were isolated from their flavour concentrates as 2,4-dinitrophenylhydrazones (DNPHs).

The raw DNPH precipitate was pre-separated by thin-layer chromatography into saturated mono-aldehyde, saturated $\beta$-ketone, monounsaturated carbonyl, cyclic and aromatic carbonyl, and dicarbonyl DNPH fractions. The first three fractions, collected from several layers, were further separated by reversed-phase high-performance liquid chromatography. Characteristic carbonyl DNPH profiles of fresh tomato, tomato purée and powder were obtained. Several individual carbonyl compounds could be tentatively identified.

The characteristic odour of tomatoes is the result of probably several hundreds of more or less volatile compounds and their interaction in, and with, the human sensory system (VAN STRATEN, 1977; PETRÓ-TURZA *et al.*, 1977). Due to the complex nature of this flavour, the number of its constituents, their widely varying polarity and concentration, no single analytical technique, including the most powerful separation methods can provide a sufficient characterization. One of the viable approaches is the extraction of the compounds and the repeated separation of the ever narrowed fractions by high efficiency separation methods, such as capillary gas chromatography and high performance liquid chromatography.

Separation of the individual carbonyls can be achieved by paper, thin-layer, classical column and gas chromatography. The number of compounds that can be separated by the first three methods is rather limited, while gas chromatographic separation of the carbonyl DNPHs is plagued by the heat-induced decomposition of the compounds tested (*e.g.* PAPA & TURNER, 1972).

High performance liquid chromatography (HPLC) proved a suitable method for the separation of carbonyl DNPHs as reviewed recently by VIGH and co-workers (1980). It was concluded that reversed phase liquid chromatography with chemically bonded octadecyl packings and aqueous methanol eluents could be used for the analysis of a number of saturated straight chain aldehyde, $\beta$-ketone and sym-ketone DNPHs. The results of this systematic investigation were used for the characterization of the volatile carbonyl profiles

of fresh tomatoes, tomato purée and powdered tomato. Whenever justified by the close correspondence of the retention volumes of the carbonyl DNPH standards and the peaks of the flavour fractions, tentative identification was also made.

## 1. Materials and methods

### *1.1. Samples*

The standards and the tomato carbonyl fractions were prepared as described in Part I (PETRÓ-TURZA & SZÁRFÖLDI-SZALMA, 1982).

### *1.2. Liquid chromatography*

Experiments were carried out by a liquid chromatograph assembled from a Model 6000A pump (WATERS ASSOCIATES, Inc., Milford, Mass. USA), *Valco* 6HPV-20 six port sampling valve equipped with a 20 $mm^3$ loop (VALCO, Houston, Tex., USA) and a 4.6 mm i.d.$\times$300 mm stainless steel column (WATERS ASSOCIATES)equipped with a full-length thermostating jacket (VIGH, 1976). The column temperature was maintained by a Type U 10 circulating water bath (MLW, Medingen, GDR). The column was slurry packed (VIGH *et al.*, 1978) with 10 $\mu$m *$\mu$Bondapak* C-18 (WATERS ASSOCIATES, Inc.). An LC 55 variable wavelength detector (PERKIN-ELMER, Norwalk, Conn., USA) set at 360 nm was used for the detection of the eluted DNPHs. Its signal was recorded on an A25 dual channel recorder VARIAN, Walnut Creek, Calif., USA). Analytical grade methanol (REANAL, Budapest, Hungary) and deionized water post-purified by extracted XAD-2 (RÖHM & HAAS, Philadelphia, Phila., USA) were used as eluents.

Eluents were filtered through Type GF/A and GF/D glass fiber filters (WHATMAN, Inc., Clifton, N. J., USA) and degassed by vacuum in an ultrasonic bath. The water content of the actual eluents withdrawn from the inlet manifold of the pump was determined by triplicate *Karl Fischer* titration using dead stop end point indication (SEYBOLD A. G., Vienna, Austria). A thermostated calibrated *Schellbach* burette, equipped at its bottom with a specially constructed inlet was used to determine the precise elution volume of the peaks. Both the standards and the flavour carbonyl fractions were freshly dissolved in the eluent [79.34% (v/v) methanol, 20.66% (v/v) water].

## 2. Results

The retention volume and the capacity factor ($k'$), defined as

$$k' = \frac{V_R - V_0}{V_0}$$

where $V_R$ is the retention volume of the eluted compound and $V_0$ is the dead volume of the column, are presented in Tables 1, 2 and 3 for the aliphatic mono-aldehyde DNPHs, aliphatic monoketone DNPHs and mono-unsaturated carbonyl DNPHs, respectively.

Table 1

*The retention volume and k' value of the ketone DNPH standards*

| Ketone-DNPH | $V_R$ (cm³) | $k'$ |
|---|---|---|
| n-$C_3$-2-one | 7.84 | 1.47 |
| n-$C_4$-2-one | 9.72 | 2.07 |
| n-$C_5$-2-one | 11.79 | 2.72 |
| n-$C_6$-2-one | 14.95 | 3.72 |
| n-$C_7$-2-one | 19.57 | 5.17 |
| n-$C_8$-2-one | 26.40 | 7.33 |
| n-$C_{11}$-2-one | 71.1 | 21.43 |
| n-$C_5$-3-one | 12.27 | 2.87 |
| 4-Me-$C_5$-2-one | 13.60 | 3.29 |
| 6-Me-n-$C_7$-5-ene-2-one | 18.96 | 4.98 |
| n-$C_7$-4-one | 19.88 | 5.27 |

Eluent: 79.34% (v/v) methanol in water
Temperature: 25.0 °C
Column: 6.3 mm o.d., 4.6 mm i.d. × 300 mm *μBondapak* C-18 (10 μm)

As an example, the chromatogram of the n-aldehyde DNPH standards is shown in Fig. 1.

The carbonyl DNPH fractions of fresh tomatoes, tomato purée and powdered tomatoes were pre-separated by TLC into five raw fractions: aliphatic monoketones; aliphatic monoaldehydes; unsaturated monocarbonyls; cyclic and aromatic carbonyls; and dicarbonyls (Petró-Turza & Szárföldi-Szalma, 1982).

The first three raw fractions were further analysed by the reversed phase HPLC system described in 1.2. Each chromatogram was run in duplicate,

Table 2

*The retention volume and k′ value of the n-aldehyde DNPH standards*

| n-aldehyde-DNPH | $V_R$ (cm³) | k′ |
|---|---|---|
| $nC_1$ | 4.96 | 0.57 |
| $nC_2$ | 6.44 | 1.03 |
| $nC_3$ | 7.57 | 1.39 |
| $nC_4$ | 8.94 | 1.82 |
| $nC_5$ | 11.12 | 2.51 |
| $nC_6$ | 14.24 | 3.49 |
| $nC_7$ | 18.72 | 4.91 |
| $nC_8$ | 25.34 | 6.99 |
| $nC_9$ | 35.14 | 10.09 |
| $nC_{10}$ | 49.20 | 14.52 |
| $iC_4$ | 8.88 | 1.80 |
| $iC_5$ | 10.41 | 2.28 |

Conditions: as in Table 1

Table 3

*The retention volume and k′ value of the mono-unsaturated aldehyde DNPH standards*

| Unsaturated aldehyde-DNPH | $V_R$ (cm³) | k′ |
|---|---|---|
| Acrolein | 7.11 | 1.24 |
| Crotonaldehyde | 8.72 | 1.75 |
| 2-hexenal | 13.43 | 3.23 |

Conditions: as in Table 1

one at the highest sensitivity setting of the UV detector, the other at such a sensitivity that all major peaks remained on scale. The retention volumes of the peaks separated and their tentative identification — when possible — are indicated in the following tables.

## *2.1. Aliphatic monoketone DNPH fractions*

The chromatograms of the fresh tomato, tomato purée and powdered tomato aliphatic monoketone DNPH fractions are shown in Figs. 2–4.

The peak elution volumes and their tentative identification are summarized in Table 4.

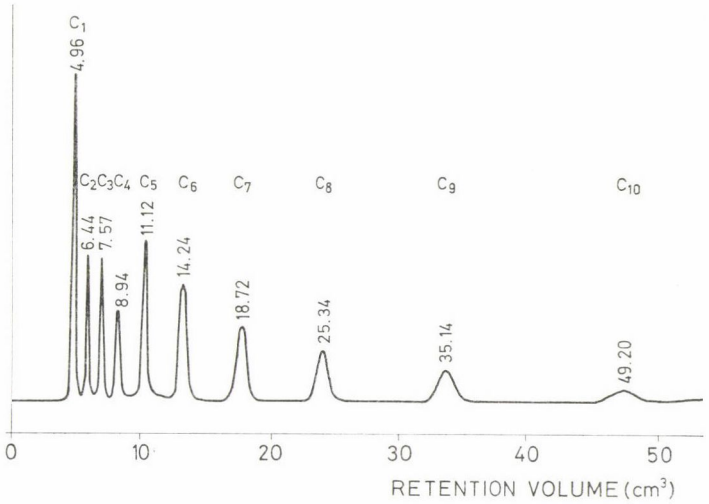


Fig. 1. Chromatogram of the n-aldehyde DNPH standards. Conditions: as in Table 1

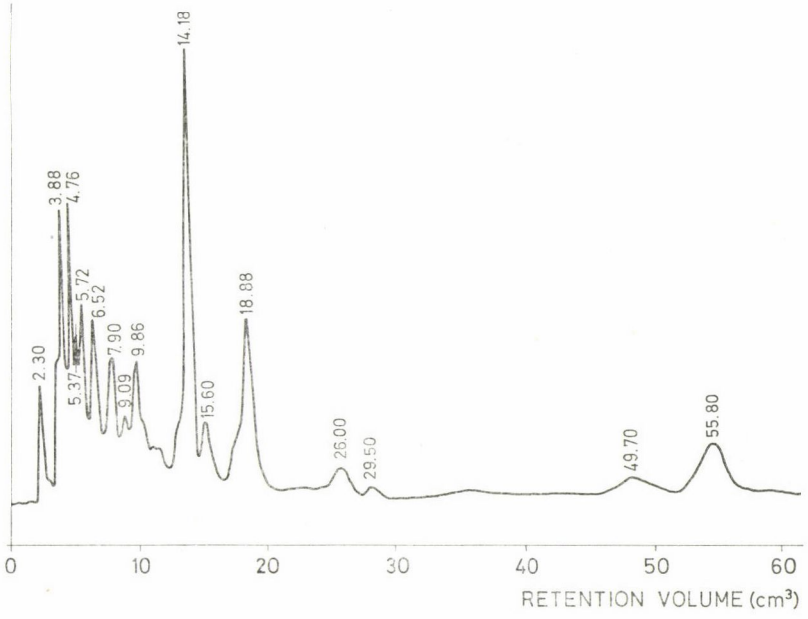


Fig. 2. Chromatogram of the ketone DNPH fraction of fresh tomatoes. Conditions: as in Table 1

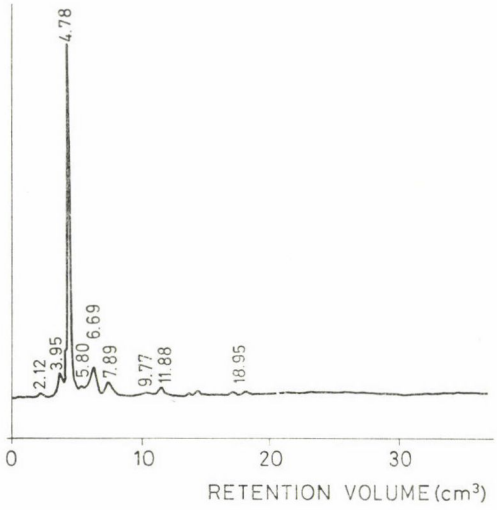


Fig. 3. Chromatogram of the ketone DNPH fraction of tomato purée. Conditions: as in Table 1

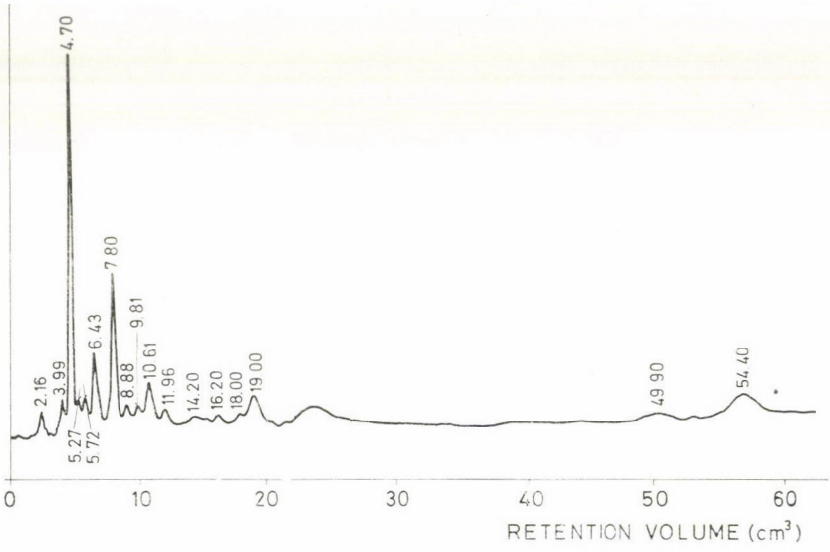


Fig. 4. Chromatogram of the ketone DNPH fraction of powdered tomato. Conditions: as in Table 1

The chromatogram of the fresh tomato ketone DNPH fraction contained many and intensive peaks. The chromatogram of the ketone DNPH fraction of the tomato purée is almost "empty", while that of the powdered tomato is again more rich. Only a very limited number of the peaks could be tentatively identified as particular keton DNPHs.

Table 4

*The elution volumes and the tentative identification of the peaks in the ketone DNPH fraction of fresh tomatoes, tomato purée and powdered tomato*

| Fresh tomato | | Tomato purée | | Powdered tomato | |
|---|---|---|---|---|---|
| $V_R$ (cm³) | tentative identity | $V_R$ (cm³) | tentative identity | $V_R$ (cm³) | tentative identity |
| — | — | 2.12 | — | 2.16 | — |
| 2.30 | — | — | — | — | — |
| 3.88 | — | 3.95 | — | 3.99 | — |
| 4.76 | — | 4.78 | — | 4.70 | — |
| 5.37 | — | — | — | 5.27 | — |
| 5.72 | — | 5.80 | — | 5.72 | — |
| — | — | — | — | 6.43 | — |
| 6.52 | — | 6.69 | — | 6.68 | — |
| 7.90 | n-$C_3$one | 7.89 | n-$C_3$one | 7.80 | n-$C_3$one |
| — | — | 8.90 | i-$C_4$ald | 8.88 | i-$C_4$ald |
| 9.09 | — | — | — | — | — |
| 9.86 | — | 9.77 | — | 9.81 | — |
| — | — | 10.70 | — | 10.61 | — |
| — | — | 11.88 | — | 11.96 | — |
| 14.18 | n-$C_6$ald | — | — | 14.20 | n-$C_6$ald |
| 15.6 | — | — | — | 16.20 | — |
| — | — | — | — | 18.00 | — |
| 18.88 | — | 18.95 | — | 19.00 | — |
| 26.0 | n-$C_8$one | 26.6 | n-$C_8$one | — | — |
| 29.5 | — | — | — | — | — |
| 49.7 | n-$C_{10}$ald | — | — | 49.9 | n-$C_{10}$ald |
| 55.8 | — | — | — | 54.4 | — |

Conditions: as in Table 1

## *2.2. Aliphatic monoaldehyde DNPH fractions*

The chromatograms of the fresh tomato, tomato purée and powdered tomato aldehyde DNPH fractions are shown in Figs. 5–7.

The peak elution volumes and their tentative identification is summarized in Table 5.

There are not as many peaks in these chromatograms as in the ketone fractions. The number of identified aldehydes is higher than that of the ketones.

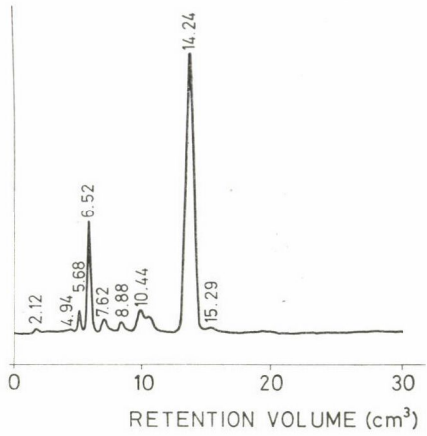


Fig. 5. Chromatogram of the aldehyde DNPH fraction of fresh tomato. Conditions: as in Table 1

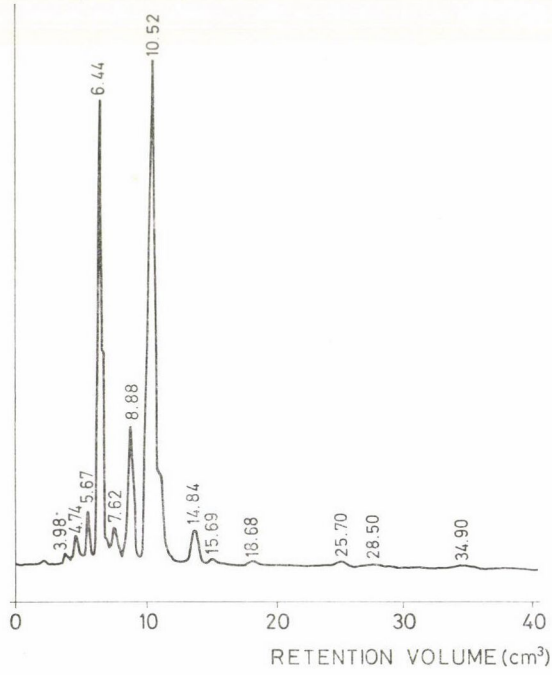


Fig. 6. Chromatogram of the aldehyde DNPH fraction of tomato purée. Conditions: as in Table 1

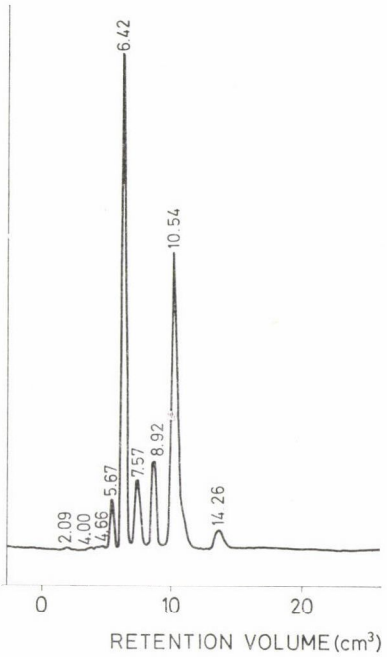


Fig. 7. Chromatogram of the aldehyde DNPH fraction of powdered tomato. Conditions: as in Table 1

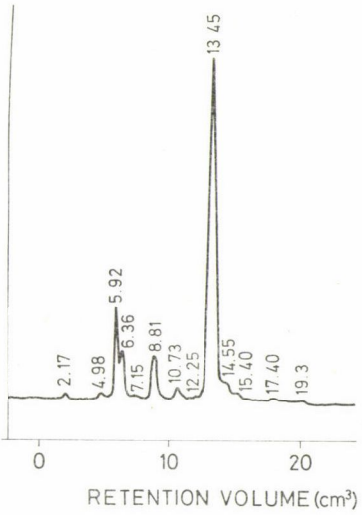


Fig. 8. Chromatogram of the unsaturated aldehyde DNPH fraction of fresh tomato. Conditions: as in Table 1

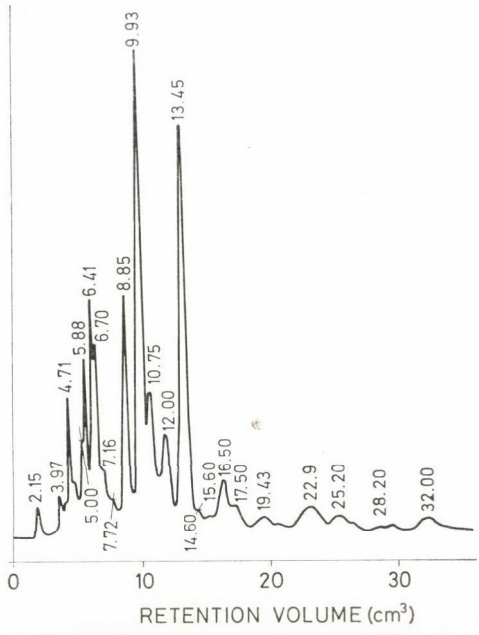


Fig. 9. Chromatogram of the unsaturated aldehyde DNPH fraction of tomato purée. Conditions: as in Table 1

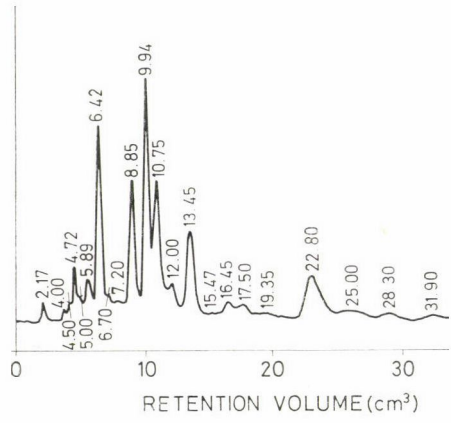


Fig. 10. Chromatogram of the unsaturated aldehyde DNPH fraction of powdered tomato. Conditions: as in Table 1

Table 5

*The elution volume and the tentative identification of the peaks in the n-aldehyde DNPH fraction of fresh tomatoes, tomato purée and powdered tomato*

| Fresh tomato | | Tomato purée | | Powdered tomato | |
|---|---|---|---|---|---|
| $V_R$ (cm³) | tentative identity | $V_R$ (cm³) | tentative identity | $V_R$ (cm³) | tentative identity |
| 2.12 | — | — | — | 2.09 | — |
| 3.96 | — | 3.98 | — | 4.0 | — |
| — | — | 4.74 | — | 4.66 | — |
| 4.94 | $C_1$ald | — | — | — | — |
| 5.68 | — | 5.67 | — | 5.67 | — |
| 6.52 | n-$C_2$ald | 6.44 | n-$C_2$ald | 6.42 | n-$C_2$ald |
| 7.62 | n-$C_3$ald | 7.62 | n-$C_3$ald | 7.57 | n-$C_3$ald |
| 8.88 | — | 8.88 | — | 8.92 | — |
| 10.44 | i-$C_5$ald | 10.52 | — | 10.54 | — |
| — | — | — | — | 13.30 | — |
| 14.24 | n-$C_6$ald | — | — | 14.26 | n-$C_6$ald |
| — | — | 14.84 | — | — | — |
| 15.29 | — | 15.69 | — | 15.66 | — |
| 18.82 | — | 18.68 | — | 18.77 | — |
| — | — | — | — | 22.77 | — |
| — | — | 23.73 | — | — | — |
| 25.27 | n-$C_8$ald | 25.7 | — | 25.4 | n-$C_8$ald |
| 28.57 | — | 28.5 | — | 28.6 | — |
| — | — | — | | 31.8 | — |
| 35.0 | — | 34.9 | — | 35.2 | |
| — | — | 39.9 | — | 40.4 | — |
| 49.3 | n-$C_{10}$ald | 49.0 | n-$C_{10}$ald | 49.5 | n-$C_{10}$ald |

Conditions: as in Table 1

## *2.3. Unsaturated monocarbonyl DNPH fractions*

The chromatograms of the fresh tomato, tomato purée and powdered tomato unsaturated DNPH fractions are shown in Figs. 8–10.

The peak elution volumes and their tentative identification are summarized in Table 6.

The unsaturated monocarbonyl DNPH fraction of the purée is the richest in peaks. The most prominent peak of the fresh tomato sample is the trans-2-hexenal, which has a characteristic "fresh fruit/vegetable" odour. Apart from the identified components there are still a number of unidentified components.

Table 6

*The elution volume and the tentative identification of the peaks in the mono-unsaturated aldehyde DNPH fraction of fresh tomatoes, tomato purée and powdered tomato*

| Fresh tomato | | Tomato purée | | Powdered tomato | |
|---|---|---|---|---|---|
| $V_R$ (cm³) | tentative identity | $V_R$ (cm³) | tentative identity | $V_R$ (cm³) | tentative identity |
| 2.17 | — | 2.15 | — | 2.17 | — |
| — | — | 3.97 | — | 4.00 | — |
| — | — | — | — | 4.50 | — |
| 4.73 | — | 4.71 | — | 4.72 | — |
| 4.98 | $C_1$ald | 5.00 | $C_1$ald | 5.00 | $C_1$ald |
| — | — | — | — | 5.70 | — |
| 5.92 | — | 5.88 | — | 5.89 | — |
| 6.36 | $C_2$ald | 6.41 | $C_2$ald | 6.42 | $C_2$ald |
| — | — | 6.70 | — | 6.70 | — |
| 7.15 | $C_3$enal | 7.16 | $C_3$enal | 7.20 | $C_3$enal |
| 7.62 | $C_3$ald | 7.72 | — | 7.88 | — |
| 8.81 | i-$C_4$ald | 8.85 | i-$C_4$ald | 8.85 | i-$C_4$ald |
| | $C_4$enal | | $C_4$enal | | $C_4$enal |
| — | — | 9.93 | — | 9.94 | — |
| 10.73 | $C_5$enal | 10.75 | $C_5$enal | 10.75 | $C_5$enal |
| 12.25 | — | 12.0 | — | 12.0 | — |
| 13.45 | $C_6$enal | 13.45 | $C_6$enal | 13.45 | $C_6$enal |
| 14.55 | — | 14.6 | — | — | — |
| 15.4 | — | 15.6 | — | 15.47 | — |
| — | — | 16.5 | — | 16.45 | — |
| 17.4 | $C_7$enal | 17.5 | $C_7$enal | 17.50 | $C_7$enal |
| 19.3 | — | 19.43 | — | 19.35 | — |
| — | — | 22.9 | $C_8$enal | 22.8 | $C_8$enal |
| — | — | 25.2 | $C_8$ald | 25.0 | $C_8$ald |
| 26.6 | — | — | — | — | — |
| — | — | 28.2 | — | 28.3 | — |
| — | — | 32.0 | — | 31.9 | — |
| — | — | 36.2 | — | — | — |
| — | — | 41.0 | $C_{10}$enal | 40.7 | $C_{10}$enal |

Conditions: as in Table 1

## 3. Conclusions

TLC allowed for the pre-separation into ketone DNPH, aldehyde DNPH, unsaturated carbonyl DNPH, cyclic and aromatic DNPH and dicarbonyl DNPH fractions of the raw carbonyl DNPH samples of fresh tomato, tomato purée and powdered tomato (PETRÓ-TURZA & SZÁRFÖLDI-SZALMA, 1982). The first three of these fractions collected from the layers were further analysed by the reversed phase HPLC system developed earlier (VIGH *et al.*, 1980). A number of peaks could be tentatively identified. The lack of the characteristic tomato odour in the case of the purée and powder samples could be substantiated by the peak intensity–pattern changes. The analytical method developed seems suitable for the objective monitoring of the results caused by changes in the processing technology aimed at the improvement of the quality of canned tomatoes.

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Addresses of the authors:

Dr. Zita VARGA-PUCHONY
Dr. Gyula VIGH
Dr. József HLAVAY

Institute for Analytical Chemistry,
University of Chemical Engineering
H-8200 Veszprém, Schönherz Z. u. 12.
Hungary

Dr. Márta PETRÓ-TURZA
Ms. Ilona SZÁRFÖLDI-SZALMA

Central Food Research Institute
H-1022 Budapest, Herman Ottó út 15.
Hungary

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# ANALYSIS OF COLD-PRESSED ORANGE OIL AND OF THE ESSENTIAL OIL PRESENT IN AQUEOUS CONDENSATES OBTAINED FROM ORANGE JUICE CONCENTRATION

J. Pino

(Received: 14 February 1980; revision received: 28 December 1980; accepted: 31 December 1980)

A comparison was made between the composition of cold-pressed essential oil and of the oil separated from the aqueous condensate during the preparation of commercial concentrated orange juice. The physical and chemical properties, the major carbonyl composition determined by quantitatively preparing and analyzing the 2,4-dinitrophenyl-hydrazine derivatives, and the column- and gas-chromatographic analysis of the volatile compounds showed some differences in oil composition. Cold-pressed essential oil generally showed higher aldehyde/ester ratios than did the essential oil present in aqueous condensate. The high content of acetaldehyde and the presence of ethyl butyrate in the essential oil present in aqueous condensate accounted for a more juice-like aroma of this oil.

During the process of concentrating orange juice, a part of the juice is evaporated, condensed and regarded as waste. This aqueous condensate containing a great amount of volatile aroma compounds is concentrated by an aroma recovery system in some industries and used as an aqueous essence to improve the quality of concentrated juice (Moshonas & Shaw, 1973; Shaw & Moshonas, 1974; Lund & Bryan, 1977).

The essential oil of the peel is mixed with the juice by the same extraction process, is volatilized at the same time, condensed with the essence and separated and floated to the top. The aqueous condensate and the essential oil represent a two-phase system in equilibrium and for this reason it would be expected that this essential oil would lack the non-volatile compounds in peel oil, but would be partially enriched in the hydrosoluble aroma compounds.

The excellent flavour quality of the oil in the condensate is similar to the juice aroma and suggested that its composition differed from that of cold-pressed essential oil. This was thought to warrant a study in order to use the essential oil present in aqueous condensate as a good flavouring agent for the food and beverage industries.

This paper reports the results of a study undertaken to determine the qualitative and quantitative composition of the essential oil present in aqueous condensate comparing with the cold-pressed essential oil.

## 1. Materials and methods

### *1.1. Samples*

Cold-pressed *Valencia* sweet orange oil was obtained from a local factory in Matanzas, Cuba. The essential oil present in aqueous condensates was obtained in the same factory during the preparation of concentrated Valencia sweet orange juice. Both samples were stored at –30 °C until analyzed.

### *1.2. Analytical methods*

*1.2.1. Physical and chemical properties.* Both oils used in this study were analyzed to be certain that they met the chemical and physical requirements as described by TAPANES (1975).

*1.2.2. Analysis of the carbonyl compounds as 2,4-dinitrophenylhydrazine derivatives.* The major carbonyl compounds were examined as follows:

Essential oils (1 g) dissolved in carbonyl-free cyclohexane (25 $cm^3$) were carefully applied to the bed of a 2,4-dinitrophenylhydrazine — ortho-phosphoric acid reaction column as described by SCHWARTZ and PARKS (1961). The mixtures were passed through the column and the non-carbonyl compounds were eluted with carbonyl-free cyclohexane. Then, the 2,4-dinitrophenylhydrazones (2,4-DNPH's) were eluted with benzene until the absorption of the eluate at 345 nm was the same as that of the eluate from a blank column developed simultaneously. Usually, the volume collected was about 40 $cm^3$.

To isolate the individual carbonyl compounds from the total 2,4-DNPH's, aliquots of the benzene eluate from the reaction column were separated by preparative thin-layer chromatography as described by BEYER and KARGL (1972) to obtain aliphatic/terpenic and aromatic fractions. Both fractions were further fractionated on *Silicagel* G plates developed with a mixture of light petroleum ether and ethyl acetate (95 : 5). Each carbonyl derivative was scraped from the TLC (thin-layer-cromatographic) plates and quantitated by its extinction coefficient. All calculations were converted to percent of total carbonyl compounds in the original 1 g of oil applied to the reaction column. The identification of individual 2,4-DNPH's was made by comparison of $R_f$ values and UV spectra with stan- pard derivatives (PINO, 1980).

*1.2.3. Separation of the oils by liquid adsorption chromatography.* Both essential oils were separated by liquid adsorption chromatography before the separation of individual compounds by gas chromatography. The columns were packed with *Silicagel* 60 (60/230 mesh, MERCK) of activity 2 to 3. The ratio sample/ /adsorbent was 1 : 20, and the oils (10 g) were eluted with n-hexane (250 $cm^3$) to separate the hydrocarbons from the oxygenated compounds eluted with diethyl ether (350 $cm^3$). Elution with each solvent was continued until no more substances could be eluted, as shown by the refractive index of the eluent. Solvents were removed from each fraction under reduced pressure in a *Rotadest* evaporator.

*1.2.4. Separation of the oils by gas chromatography.* Samples of essential oils were chromatographed on a *Packard–Becker* 419 equipped with 3 m × 0.4 cm i.d. stainless steel columns packed with either 5% polyethyleneglycol adipate or 5% SE 30 on 60/80 mesh *Chromosorb* G (BDH, England). The temperature was 80 °C initially and was raised to 200 °C at 5 °C $min^{-1}$. The flame ionization detector operated at 220 °C and the injection port was at 200 °C. The argon carrier gas flow rate was 25 $cm^3$ $min^{-1}$.

Peaks were identified by comparison of retention data on polar and nonpolar columns, and of infrared or mass spectra with those found for authentic samples.

Quantitative peak-area measurements were made with an *Autolab* 6300 digital integrator and the response factors of the main compounds were calculated to determine the percent concentration of each compound (KEULEMANS, 1959).

Preparative gas chromatography was carried out using a *Pye Unicam* 105 equipped with a 8 m × 0.9 cm stainless steel column packed with 15% *Carbowax* 20 M on 30/60 mesh *Chromosorb* W (BDH, England). The column temperature was 200 °C. The flame ionization detector operated at 220 °C and the injection port was at 200 °C. The argon carrier gas flow rate was 100 $cm^3$ $min^{-1}$.

The isolated compounds were analyzed by spectrometric techniques. The infrared spectra were registered in a *Carl Zeiss* UR 20 spectrophotometer and the mass spectra in a *Hitachi* RMU 6D mass spectrometer.

## 2. Results

Table 1 lists the physical and chemical properties of the cold-pressed essential oil and the oil present in aqueous condensate.

The analysis of the data (Table 1) reflects the chemical difference among the oils of some oxygenated compounds. From the standpoint of flavour, the

Table 1

*Physical and chemical properties of the cold-pressed essential oil and the essential oil present in the aqueous condensate obtained from orange juice concentration*

| Property | Cold-pressed oil | Essential oil present in aqueous condensate |
|---|---|---|
| Refractive index ($n_D^{20}$) | 1.4794 | 1.4790 |
| Relative density ($d_{20}^{20}$) | 0.8446 | 0.8594 |
| Aldehydes % (wt/wt)[a] | 0.96 | 0.90 |
| Esters % (wt/wt)[b] | 0.88 | 1.47 |

[a] determined as decanal by the hydroxylamine method reported by TAPANES (1975)
[b] determined as geranyl acetate by the volumetric method reported by TAPANES (1975)

ratios of total aldehydes and esters are very important since most of the flavour of citrus fruits comes from these compounds. The essential oil present in aqueous condensate has a much lower aldehyde–ester ratio than the cold-pressed oil. This is a result of the higher percentage of esters and is partially responsible for the more juice-like aroma of the essential oil present in the aqueous condensate.

In order to know the composition of the major carbonyl compounds, they were determined quantitatively by analyzing as 2,4-DNPH's. Results are summarized in Table 2.

Table 2

*Concentration of the major carbonyl compounds in cold-pressed essential oil and the essential oil present in aqueous condensate obtained from orange juice concentration*
(Means and standard deviations of triplicate measurements)

| Carbonyl compound | Cold-pressed oil[a] | Essential oil present in aqueous condensate[a] |
|---|---|---|
| Acetaldehyde | trace | 15.3 ± 2.3 |
| Hexanal | trace | 0.4 ± 0.1 |
| Octanal | 18.2 ± 1.8 | 12.5 ± 1.9 |
| Nonanal | 10.2 ± 2.1 | 8.2 ± 2.0 |
| Decanal | 30.9 ± 2.6 | 23.5 ± 3.2 |
| Undecanal | 9.1 ± 1.8 | 10.0 ± 2.2 |
| Dodecanal | 8.5 ± 1.3 | 4.1 ± 1.0 |
| Tetradecanal | 4.6 ± 1.0 | 8.0 ± 1.2 |
| Neral/geranial | 10.8 ± 0.8 | 10.3 ± 0.6 |
| Furfural | trace | trace |

[a] concentration as % (w/w) for total carbonyl compounds

The major carbonyl compounds of both essential oils are aldehydes. They account for 92.3% of the total carbonyl compounds. The other carbonyls are more complex and the identity of these compounds is being studied. The essential oil present in the aqueous condensate has a greater acetaldehyde content compared with the cold-pressed oil. It is expected that the presence of acetaldehyde contributes to the juice-like aroma of this essential oil.

A typical gas chromatogram of both essential oils is shown in Fig. 1a, and 1b. Results are summarized in Table 3.

Eighteen compounds identified are listed in order of their GC-retention times on polyethyleneglycol adipate. Means of identification of each compound and quantitative estimates are given.

The qualitative analysis shows that there are some differences between the essential oils. Thus, ethyl butyrate was present only in the essential oil of the aqueous condensate. The presence of this ester in the oil can be explained

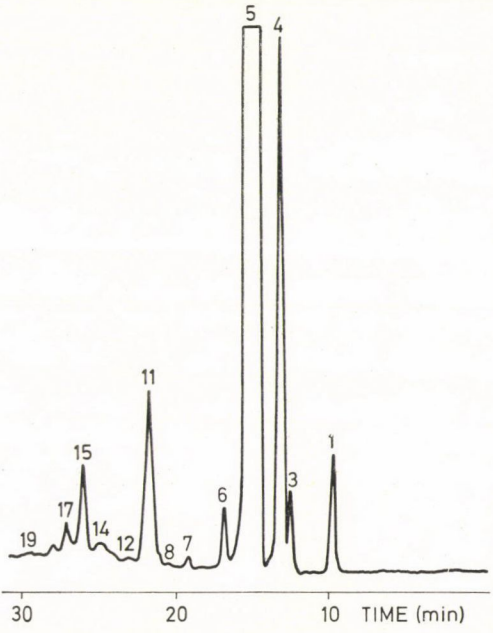


Fig. 1a. Separation by gas chromatography of the cold-pressed sweet orange oil on polyethyleneglycol adipate column pack
Injector temperature: 200 °C; detector temperature: 220 °C; rate of the carrier gas: 25 $cm^{-3}$ $min^{-1}$. The temperature program was 80 °C – 200 °C at 5 °C $min^{-1}$. Peak assignations are in Table 3

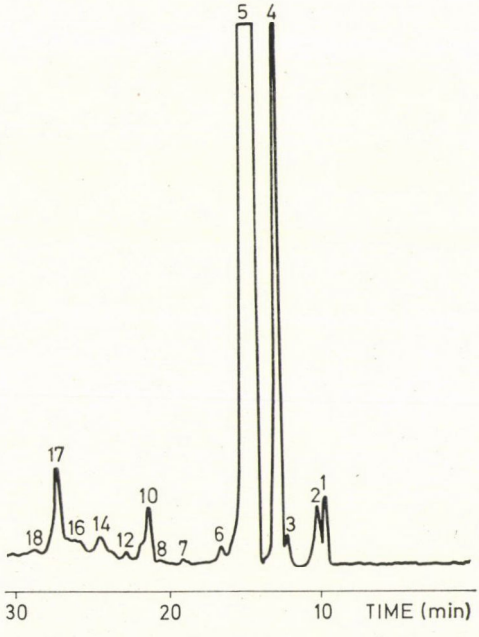


Fig. 1b. Separation by gas chromatography of the essential oil present in aqueous condensate from orange juice concentration on polyethyleneglycol adipate column pack
Conditions see in Fig. 1a

as the result of the partitioning of volatile hydrosoluble compounds between the aqueous condensate and the oil. Another difference between the two essential oils accounts for the higher percent of aliphatic aldehydes in the cold-pressed oil than in the oil present in aqueous condensates.

Table 3

*Qualitative and quantitative analysis of cold-pressed essential oil and the essential oil present in aqueous condensate obtained from orange juice concentration*
(Means and standard deviations of triplicate measurements)

| Peak Nr. | Compound | Concentration % (w/w) | | Methods of identification |
|---|---|---|---|---|
| | | Cold-pressed oil | Essential oil present in aqueous condensate | |
| 1 | α-pinene | 0.9 ± 0.01 | 0.4 ± 0.01 | GC, IR, MS |
| 2 | ethyl butyrate | — | 0.4 ± 0.01 | GC, IR |
| 3 | sabinene | 0.6 ± 0.01 | 0.2 ± 0.01 | GC |
| 4 | myrcene | 2.9 ± 0.02 | 2.9 ± 0.02 | GC, IR, MS |
| 5 | limonene | 88.0 ± 0.40 | 93.4 ± 0.37 | GC, IR, MS |
| 6 | octanal | 0.6 ± 0.01 | trace | GC |
| 7 | nonanal | 0.1 ± 0.01 | 0.1 ± 0.01 | GC |
| 8 | unknown | trace | — | — |
| 9 | furfural | 2.5 ± 0.02 | 0.7 ± 0.01 | GC |
| 10 | decanal | | | GC |
| 11 | linalool | | | GC, IR, MS |
| 12 | undecanal | 0.1 ± 0.01 | 0.1 ± 0.01 | GC |
| 13 | terpinen-4-ol | 0.3 ± 0.01 | 0.1 ± 0.01 | GC, IR |
| 14 | unknown | 0.6 ± 0.01 | 0.5 ± 0.01 | — |
| 15 | α-terpineol, | 1.5 ± 0.02 | 0.4 ± 0.01 | GC, IR, MS |
| | valencene | | | GC, IR, MS |
| 16 | neral | 0.1 ± 0.01 | 0.1 ± 0.01 | GC, IR |
| 17 | geranyl acetate | 0.8 ± 0.02 | 0.8 ± 0.02 | GC, IR |
| | geranial | | | GC |
| 18 | geraniol | 0.4 ± 0.01 | trace | GC, IR |
| 19 | unknown | 0.4 ± 0.01 | — | — |

GC, IR, MS: identification by gas chromatography, infrared and mass spectra

## 3. Conclusions

Summarizing our results, we may say that the ratio aldehyde–ester in the essential oil present in the aqueous condensate is much lower than in cold-pressed essential oil as a result of the higher percentage of esters and is partially responsible for the juice-like aroma of this essential oil.

We found some quantitative differences in the volatile composition of the essential oils. Ethyl butyrate and acetaldehyde were present only in the essential oil of the aqueous condensate. It is assumed that the presence of these compounds contributes to the juice-like aroma of this essential oil and warrants its use as an excellent flavouring agent for the food and beverage industries.

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Address of the author:

JORGE PINO — Department of Fruits and Vegetables,
National Center of Scientific Research
Apartado 6880, Habana. Cuba

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# ATTEMPTS TO UTILIZE WHEY FOR THE PRODUCTION OF YEAST PROTEIN

## III. EFFECT OF SOME VITAL GROWTH FACTORS

H. NOUR EL-DIEN and A. HALÁSZ

(Received: 29 April 1980; revision received: 23 January 1981; accepted 31 January 1981)

Vital growth factors as vitamins B, amino acids and yeast extract on *S. fragilis* were investigated in synthetic and whey medium. It was found that only yeast extract was effective in increasing protein content and yield coefficient. Whey contains stimulating factor other than amino acids, vitamins B and nucleic acid bases which also seem to be present in yeast extract.

In view of the world shortage of dietary protein, there is an urgent need to improve the quality of the diet of the majority of the human race. One solution is the development of new sources of high-quality protein.

The production of microorganisms, yeasts in particular, by fermentation processes is an important new venture to increase the resources of high-quality protein. Whey fermentation with suitable yeasts is now possible on a commercial scale, particularly where there are problems of waste disposal. The literature on whey utilization has been reviewed previously (NOUR EL-DIEN & HALÁSZ, 1981; 1982).

In previous studies, NOUR EL-DIEN and HALÁSZ (1981, 1982) reported that addition of biotin to whey had no effect on the growth of yeast. They thought that the whey medium itself contains enough vital growth factors.

Thus, the aim of this study was to study the effect of vital growth factors, such as amino acids, vitamins B, bases of nucleic acids and yeast extract on the growth of *Saccharomyces fragilis*.

### 1. Materials and methods

The microorganisms, culture media, cultivation methods, determination of cell growth, lactose, protein and nucleic acids content were the same as described previously (NOUR EL-DIEN & HALÁSZ, 1981; 1982).

Moreover, fermentation in shaken culture was used. One hundred $cm^3$ of the fermentation broth were inoculated by inoculum cells in 500 $cm^3$ Erlenmeyer flasks to set a cell concentration of about 0.6 g $dm^{-3}$ then incubated on a shaker at 330 rpm for 20 h at 30 °C.

The replating technique and a modified disc diffusion method (COLLINS, 1967; NARASINHACHARI & RAMACHANDRAN, 1967) were used to study the effect of amino acids, nucleic acid bases and yeast extract (by using 15 $\mu$l from solution 9 mg $cm^{-3}$ from each amino acid in Table 4 and bases of nucleic acid "adenine, guanine, uracil and thymine").

## 2. Results

### *2.1. Effect of vitamins B and yeast extract*

This experiment was carried out in shaken cultures, in 500 $cm^3$ Erlenmeyer flasks. The latter contained 100 $cm^3$ of the following media: (1) mineral medium (Table 1) with 2% lactose, (2) mineral medium with 2% lactose, supplemented with vitamins B 1 $cm^3$ $dm^{-3}$ (from a vitamins B mixture, see page 130, Table 5), (3) mineral medium supplemented with 0.5% yeast extract powder (DIFCO), (4) whey medium with 2% lactose, supplemented with nitrogen and phosphorus salts at the same percent as in mineral medium.

Table 1

*Composition of mineral medium for yeast cultivation*

| Component | Quantity |
|---|---|
| $MgSO_4$ | 1.0 g |
| $KH_2PO_4$ | 1.4 g |
| $Na_2HPO_4 \cdot 12\,H_2O$ | 8.0 g |
| NaCl | 10.0 g |
| $(NH_4)_2SO_4$ | 40.0 g |
| Distilled water to make | 10.0 liter |

Table 2

*Vitamin composition of whey powder*
(WASSERMAN, 1961)

| Vitamin | Quantity | |
|---|---|---|
| | Average | Extreme values |
| Biotin ($\mu$g per 100 g) | 29.00 | 3–150 |
| Riboflavin (mg per 100 g) | 2.10 | 0.5–6.8 |
| Niacin (mg per 100 g) | 0.30 | 0.3–2.2 |
| Pantothenic acid (mg per 100 g) | 0.10 | 2.2–9.0 |
| Pyridoxine ($B_6$) (mg per 100 g) | 0.27 | 0.05–1.5 |
| Thiamin (mg per 100 g) | 0.86 | 0.4–1.5 |

HARJU and co-workers (1976), SMITH and BULL (1976), CASTILLO and SÁNCHEZ (1978), reported that addition of vitamins B is necessary for good yields of yeast, and yeast extract can be used as a vitamin supplement.

Our results, presented in Table 3, indicate that the propagation of *S. fragilis* on whey medium gave highest net dry weight, lactose utilization and yield coefficient (3.33 mg $cm^{-3}$, 100% and 17.52%, respectively). This may be due to the whey which contains substantial amounts of vitamins (Table 2). However, supplementation of mineral medium with yeast extract increased both net dry weight and lactose utilization, also yield coefficient (3.21 mg $cm^{-3}$, 100% and 16.90%, respectively). But when the vitamin complex was used to replace yeast extract, net dry weight, lactose utilized and yield coefficient also increased (1.77 mg $cm^{-3}$, 68.42% and 13.62%, respectively) however not to the same level as with yeast extract or as with whey.

Table 3

*Effects of vitamins B and yeast extract on the growth of* S. fragilis *as dry weight and residual lactose in shaken cultures*

| Medium | 0 h | | 20 h | | Net dry weight (after 20 h) | Lactose utilized (after 20 h) | Yield coefficient | Nucleic acid | Protein content | | PNC |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | dry weight | lactose | dry weight | lactose | | | | | Crude protein (N × 6.25) | True protein [(TN-$N_{RNA}$) × 6.25] | |
| | mg $cm^{-3}$ | | | | | % | | | | | |
| 1 | 0.48 | 19 | 1.050±0.05 | 11.50 | 0.57 | 39.47 | 7.6 | 7.7 | 45.8 | 37.94 | 16.8 |
| 2 | 0.48 | 19 | 2.250±0.05 | 6.00 | 1.77 | 68.42 | 13.62 | 7.9 | 45.7 | 37.63 | 17.2 |
| 3 | 0.48 | 19 | 3.690±0.05 | 0.00 | 3.21 | 100.00 | 16.90 | 9.8 | 49.2 | 39.69 | 19.9 |
| 4 | 0.48 | 19 | 3.810±0.05 | 0.00 | 3.33 | 100.00 | 17.52 | 7.0 | 44.5 | 37.38 | 15.7 |

Media: (1) Mineral medium + 2% lactose
(2) Mineral medium + 2% lactose + vitamins B, 1 $cm^3$ $dm^{-3}$
(3) Mineral medium + 2% lactose + 0.5% yeast extract powder (DIFCO)
(4) Whey medium with 2% lactose + NIP in the same percent as in mineral medium

Yield coefficient is milligram cell produced per milligram lactose utilized multiplied by 100 (CASTILLO & SÁNCHEZ, 1978)

$$\text{PNC (Protein nitrogen coefficient)} = \frac{\text{NAN (Nucleic acid nitrogen)}}{\text{total N}} \times 100;$$ (POKROVSKY, 1975)

On the other hand, the nucleic acid content of dry harvested yeasts which were grown on mineral media, was increased from 7.7 to 9.8 per cent by increasing the net dry weight from 0.57 to 3.21 mg $cm^{-3}$ (according to VANANUVAT & KINSELLA, 1975). On the other hand, the nucleic acid content of yeast which had been grown on whey medium was lower (7.0%), while the net dry weight was higher (3.33 mg $cm^{-3}$) than in the mineral medium.

According to the results (Table 3), there were no significant differences in crude protein content between the yeasts grown in whey and in mineral

medium or in mineral medium supplemented with vitamin B. Surprisingly, the crude protein of the yeasts grown on mineral media supplemented with yeast extract was higher (49.3%), than in any other investigated case.

On the other hand, by calculating the protein nitrogen coefficient (PNC) which reflects the percentage of nucleic acid nitrogen (NAN) per total nitrogen of the biomass, we realised that the yeast grown on whey had a lower value (15.73%), while the yeast grown in mineral media supplemented with yeast extract had a higher value (19.92%).

Thus, from these results, we think that the whey complemented with yeast extract powder (DIFCO) contains also other growth factors than vitamins B.

Therefore, the yeast extract powder was analyzed, with an amino acid analyzer, to establish the amino acid content of yeast extract powder (Table 4).

Table 4

*Analysis of yeast extract powder (DIFCO) by amino acid analyzer*

| Amino acid | % | Amino acid | % |
|---|---|---|---|
| 1. Lysine | 4.997 | 9. Glycine | 3.133 |
| 2. Histidine | 1.473 | 10. Alanine | 5.113 |
| 3. Arginine | 3.097 | 11. Valine | 3.990 |
| 4. Asparagine | 6.663 | 12. Methionine | 0.930 |
| 5. Threonine | 2.960 | 13. Isoleucine | 3.483 |
| 6. Serine | 3.153 | 14. Leucine | 4.273 |
| 7. Glutamic acid | 13.083 | 15. Tyrosine | 1.273 |
| 8. Proline | 3.573 | 16. Phenylalanine | 2.910 |

### *2.2. Effect of amino acids and nucleic acid bases on the growth of* S. fragilis

This experiment was carried out by using the replica plating technique. The media were as follows:

(1) mineral medium + 2% lactose
(2) mineral medium + 2% lactose + L-lysine
(3) mineral medium + 2% lactose + DL-glutamine
(4) mineral medium + 2% lactose + DL-methionine
(5) whey medium with 2% lactose
(6) whey medium with 2% lactose + 0.5% yeast extract powder.

The amino acids were added in equivalent concentrations as in yeast extract powder (Table 4). The results (Fig. 1) indicate that the growth in whey and in the whey medium supplemented with yeast extract was better than in the other media.

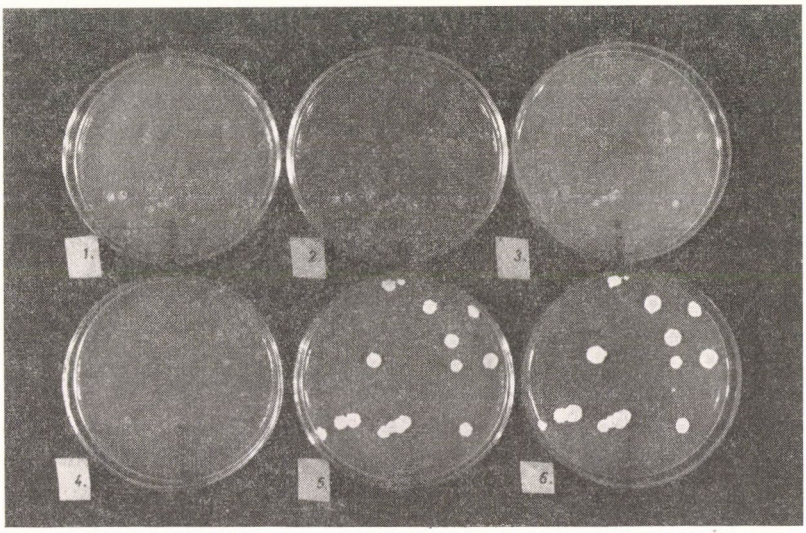


Fig. 1. Effect of amino acids and yeast extract on the growth of *S. fragilis*
(1) mineral medium,
(2) mineral medium supplemented with L-lysine,
(3) mineral medium supplemented with DL-glutamic acid,
(4) mineral medium supplemented with DL-methionine,
(5) whey medium,
(6) whey medium supplemented with yeast extract

This experiment was repeated, by addition of basis of nucleic acid such as, adenine, guanine, cytosine and uracil, at equivalent amounts as by supplementing yeast extract powder. The amino acids mentioned previously and the nucleic acid bases were added to the media separately and together, as they might cause a synergistic effect. The results did not show any effect of the addition of bases of RNA or of amino acids.

We used the disc-diffusion method [applying solutions 9 mg $cm^{-3}$ of each amino acid (Table 4) and nucleic acid bases adenine, guanine, uracil and thymine separately]. We obtained the same results as previously, except for the very slight effect of alanine, cystein and histidine. GLÄTTLI and BLANC (1974) reported that the addition of alanine was necessary for good yeast growth.

*2.3. Effect of vital growth factors on the growth of* S. fragilis *using column fermentors*

This experiment was carried out in six column fermentors containing:
(1) mineral medium + 2% lactose;
(2) mineral medium + 2% lactose + 6 mg $dm^{-3}$ from each amino acid mentioned in Table 4;
(3) mineral medium + 2% lactose + 1 $cm^3$ $dm^{-3}$ from the mixture of vitamins B in Table 5;

(4) mineral medium + 2% lactose + 0.5% yeast extract powder (DIFCO);
(5) whey medium with 2% lactose + N + P in the same concentration as in the mineral medium;
(6) whey medium with 2% lactose + N + P in the same concentration as in the mineral medium + 0.5% yeast extract powder (DIFCO).

Comparing the results in Table 6 with those in Table 3 for the same media and growth parameters, it is clear that, there are highly significant differences, possibly due to the fact that, in column propagators, the oxygen absorption rate was much higher than in shaken cultures (this phenomenon will be studied next).

Table 5

*Mixture of vitamins B*

| Quantity (mg) | Vitamin |
|---|---|
| 0.2 | Folic acid |
| 0.2 | Biotin |
| 40 | Ca-pantothenate |
| 200 | Inositol |
| 40 | Niacin |
| 20 | P-aminobenzoic acid |
| 40 | Pyridoxin-HCl |
| 40 | Aneurin-HCl (Thiamine) |
| 20 | Riboflavin |
| 100 | Distilled water |

*2.3.1. The specific growth rate.* From results given in Table 6 and Fig. 2, it was obvious that the addition of yeast extract to either mineral or whey media increased the specific growth rate (from 0.131 to 0.401 $h^{-1}$ and from 0.303 to 0.450 $h^{-1}$, respectively). This result agrees with that obtained by HARJU and co-workers (1976), CASTILLO and SÁNCHEZ (1978), NOUR EL-DIEN and HALÁSZ, (1981, 1982).

Supplementation of amino acids (Medium No. 2) or vitamins B (Medium No. 3) instead of yeast extract, resulted in the specific growth rate remaining very low (0.134 $h^{-1}$ and 0.146 $h^{-1}$). This is in contrast to the findings of HARJU and co-workers (1976), who reported that addition of a vitamin complex increased the specific growth rate to the same level as yeast extract.

On the other hand, the specific growth rate reflects on the generation time. Therefore, the minimum in generation time was estimated when yeast extract was added to whey medium (1.73 g). But supplementation of mineral medium with vitamins B has slightly decreased the generation time (from

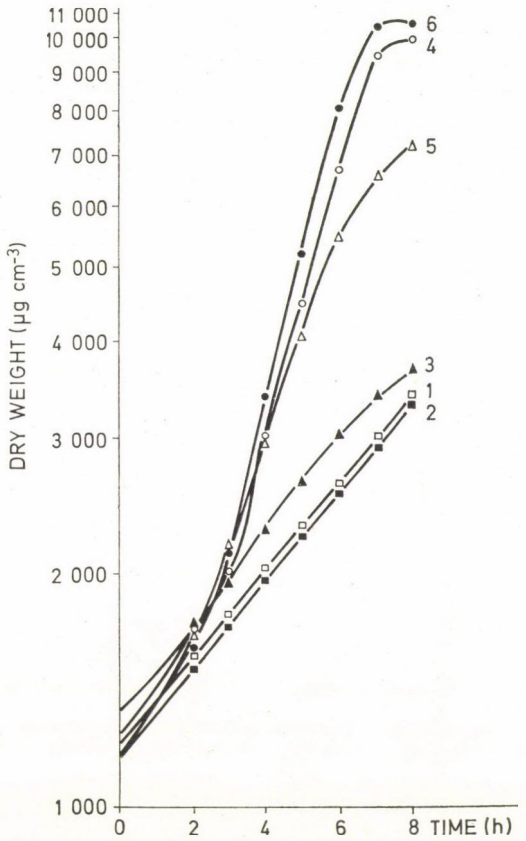


Fig. 2. Effect of vital growth factors (amino acids, vitamins B and yeast extract) on the growth rate of *S. fragilis*

5.29 to 4.75 h), while addition of amino acids to the mineral medium caused no significant difference in generation time (5.17 h).

*2.3.2. The yield coefficient and lactose utilization.* It is clear from the results (Table 6 and Figs. 2–3) that the growth of *S. fragilis* showed a high yield when the mineral or the whey media were supplemented with yeast extract (36.87% and 45.02%, respectively). Therefore, a high efficiency in the conversion of lactose to cell material could be demonstrated. However, addition of amino acids or vitamins B to the mineral medium has no effect on the yield coefficient. While HARJU and co-workers (1976) as well as SMITH and BULL (1976) reported good yields for the same yeast grown on medium supplemented with vitamins B.

*2.3.3. The nucleic acid and protein content of* S. fragilis *and its relation to the protein nitrogen coefficient.* The protein nitrogen coefficient (PNC) is

Table 6

*Effect of vital growth factors (amino acids, vitamins B) and yeast extract on the growth parameters of* S. fragilis *in column fermentors*

| Medium | Specific growth rate ($k$) | Generation time | Net dry weight (after 8 h) | Lactose utilized (after 8 h) | Yield coefficient | Nucleic acid | Protein content | | Protein nitrogen coefficient (PNC) |
|---|---|---|---|---|---|---|---|---|---|
| | | | | | | | Crude protein ($N \times 6.25$) | True protein $[(TN\text{-}N_{RNA}) \times 6.25]$ | |
| | $h^{-1}$ | h | mg $cm^{-3}$ | % | % | % | % | % | |
| 1 | 0.131±0.011 | 5.29 ± 0.50 | 2.17 ± 0.27 | 45.53±0.57 | 17.44±0.43 | 8.70 ± 0.04 | 45.62±0.28 | 36.74±0.23 | 19.45±0.18 |
| 2 | 0.134±0.013 | 5.17 ± 0.55 | 2.15 ± 0.24 | 51.06±0.57 | 17.92±0.45 | 8.60 ± 0.04 | 44.80±0.24 | 36.05±0.23 | 19.60±0.17 |
| 3 | 0.146±0.020 | 4.75 ± 0.70 | 2.32 ± 0.35 | 55.32±0.60 | 17.85±0.46 | 8.60 ± 0.05 | 46.77±0.25 | 37.99±0.24 | 18.85±0.16 |
| 4 | 0.401±0.036 | 1.73 ± 0.14 | 8.59 ± 0.48 | 97.87±0.94 | 36.87±0.48 | 9.70 ± 0.05 | 48.85±0.25 | 38.94±0.28 | 20.20±0.20 |
| 5 | 0.303±0.015 | 2.29 ± 0.12 | 5.97 ± 0.37 | 99.03±1.07 | 29.12±0.83 | 7.00 ± 0.03 | 44.50±0.24 | 37.35±0.28 | 16.01±0.18 |
| 6 | 0.450±0.018 | 1.54 ± 0.62 | 9.23 ± 0.64 | 99.03±1.08 | 45.02±1.03 | 8.80 ± 0.05 | 49.67±0.25 | 40.68±0.29 | 18.11±0.18 |
| $F$-test | H.S. | H.S. | H.S. | H.S. | H.S. | H.S. | H.S. | H.S. | H.S. |
| $LSD_{0.5\%}$ | 0.021 | 0.180 | 0.631 | 1.78 | 1.34 | 0.14 | 0.99 | 0.71 | 0.58 |

Media: (1)Mineral medium + 2% lactose (2) Mineral medium + 2% lactose + amino acids (3) Mineral medium + 2% lactose + B vitamins (4) Mineral medium + 2% lactose + 0.5 yeast extract (5) Whey medium + 2% lactose (6) Whey medium + 2% lactose + 0.5% yeast extract

Yield coefficient is milligram cell produced per milligram lactose utilized multiplied by 100 Generation time = ln 2/K (specific growth rate)
H.S. = highly significant difference at $P \geq 99\%$ probability level; $LSD_{0.5}\%$ = least significan difference ($P \geq 95\%$)

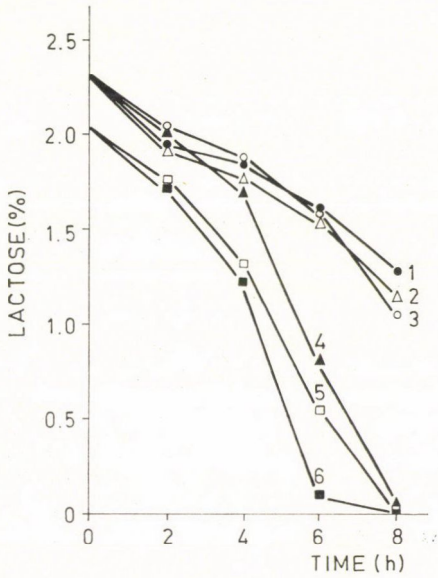


Fig. 3. Effect of vital growth factors (amino acids, vitamins B and yeast extract) on lactose consumption

expressed as percentage of nucleic acid nitrogen per total biomass nitrogen. Thus, from the results in Table 6, the nucleic acid content of yeast was higher when mineral and whey media were supplemented with yeast extract (9.7 and 8.8%, respectively). However, the same nucleic acid content was found in yeast grown in mineral medium or in mineral medium supplemented with amino acids or with vitamins B (8.6–8.7%). From the results in Table 6, an addition of yeast extract to mineral or whey media improved the growth rate of yeast, and the nucleic acid content is affected by the growth rate.

On the other hand, an addition of yeast extract to mineral and whey media appeared to improve slightly the protein content of yeasts, but there was a very slight difference due to the addition of vitamins B.

By calculating the protein nitrogen coefficient as a biological value indicator for dried harvested yeasts (Table 6) it can be seen that the lowest PNC (16.01%) was obtained when the yeast was cultivated on whey medium. The addition of yeast extract to whey increased the growth rate and at the same time the RNA content. But even in that case the PNC had a value lower than the others.

## 3. Conclusions

Growth of *Saccharomyces fragilis* on whey medium was better than on mineral medium. It is important to add yeast extract to whey medium. The cultivation conditions were better, especially the yield coefficient (45.02%),

and the true protein content (40.68%). The generation time (1.54 h) as well as the protein nitrogen coefficient (18.11) were lower.

However, when amino acids, vitamins B and nucleic acid bases were used, instead of yeast extract, the fermentation data did not improve to the same level as with yeast extract or in whey medium.

Thus, we think that the whey medium contains some stimulating factors other than amino acids, vitamins B and nucleic acid bases, which improve the growth of *S. fragilis*, and these vital growth factors also seem to be present in yeast extract.

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Address of the authors:

DR. HASSAN NOUR EL-DIEN*
DR. ANNA HALÁSZ

Central Food Research Institute
H-1022 Budapest, Herman Ottó út 15.
Hungary

* Present address:

Dairy Department,
Faculty of Agriculture,
Tanta University
Kafr el-sheikh, Alexandria.
Egypt

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# DETERMINATION OF RIPENESS IN TOMATO JUICE AND PAPRIKA PERICARP BY SPECTROPHOTOMETRY

GY. HORVÁTH and L. ANDRÉ

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Tomatoes and paprika may be considered completely ripe when the green colour of chlorophyll is suppressed in the colouring matter by lycopene in tomatoes and a wide range of carotenoids in paprika. Since ripeness can not be established visually with absolute certainty a new conception of determination was worked out based on the measurement of absorbance in the fruits. It is suggested to measure absorbance at 588 and 680 nm. The correlation coefficients ($r$) with the *Hunter* L a/b value are: for tomatoes 0.963 and for paprika 0.753.

Colour is one of the most important quality characteristics of tomato concentrates and ground paprika. Colour depends in both on carotenoid pigments which reach their maximal level at the stage of full maturity. On the other hand, determination of full maturity facilitated objective quality assessment of the raw material and its acceptance at the factory according to quality. Developments in processing technologies also require the use of objective methods of quality assessment.

In recent years tomato delivery in Hungarian factories has greatly changed. Decreasing amounts of fruits are delivered in whole to the factories; the fruit is pulped at juice stations in the neighbourhood of the tomato fields. This change, besides influencing the organization of processing, requires the extension of quality parameters. In order to be able to guarantee an end product of good colour, it is necessary to determine not only the water soluble solids and sand content but also the colour of the raw juice, has to be established. The water soluble solids content is in close correlation with maturity, yet, it alone does not suffice to describe raw juice, as this quality characteristic is not closely related to the colour of the concentrate.

In the last decade, paprika production has increased considerably in Hungary. Labour shortage forced farms to adopt machine harvesting. In Central Europe, weather conditions are rather extreme during the ripening period of paprika. Early frost may occur any time, thus, it is very important to posses objective methods for the determination of the stage of fruit maturity. Colour is one of the most important quality factors of the ground product on the market. Only fruits harvested at full maturity guarantee good colour. Consequently objective qualification requires colour evaluation and delivery based on quality.

The two raw materials described above are highly different. One is a fibrous pulp, the other a solid skin tissue. We consider, however, that the method developed to determine maturity may be used successfully with both raw materials. There are numerous methods to assess tomato maturity from visual colour sorting to instrumental measurement of maturity. Objective instrumental methods now used can be divided into two major groups. In the first case, the light passes through the whole fruit and it is the ratio of absorption at two different wavelength that gives information on the ripeness of the fruit, *i.e.* about its inner colour (BIRTH *et al.*, 1957; BIRTH & NORRIS, 1958; WATADA *et al.*, 1976). The best known of this methods are *Rheobiospect* and *Hortispect*. KRIVOSHIEV (1974) also solved the problem of tomato sorting according to maturity by using the ratio of light absorption in the fruit. Sorting machines based on this principle have successfully been working for years in Plovdiv, Bulgaria.

The other method used light reflection to determine maturity. *Agtron*-E is the best known instrument utilizing this principle. Measurements are carried out on fruit cut in half; maturity is expressed on a percentage scale based on empirical principle (MACKINNEY, 1962).

Methods based on reflection are used only to evaluate maturity or colour in the pulp. *Agtron*-E is well adapted for measuring the colour of pulp as well. *Tomacolor* developed at the RESEARCH INSTITUTE OF THE CANNING AND PAPRIKA INDUSTRIES (Budapest) is also based on reflection (BONTOVITS *et al.*, 1978; BONTOVITS, 1979). Both instruments measure the ratio of reflectances at two wavelengths.

Apart from the instruments mentioned above, the $a_L/b_L$ ratio derived from *Hunter*'s colour system has been the standard method in several countries to evaluate tomato juice colour. This is also based on the measurement of reflection, but the ratio is calculated by using the whole visible spectrum according to the principles of colorimetry (GOOSE & BINSTED, 1973).

Very few data are available on the objective quality assessment of paprika. Beside traditional, subjective sorting, little attention was given to this problem (PÜSPÖK, 1979). The objective quality assessment of paprika is more difficult than that of tomato pulp, due to the structure of the skin of paprika. It is not thick enough for the measurement of reflection, because the light passes through it. The difficulty can not be solved by placing several layers one upon the other. A homogenous layer can not be obtained, because the cells are of different size and air content.

On the basis of our experiments, we consider the solution of this problem to be found in light transmission systems. Ways and instruments must be found to evaluate light transmission in pulp or juice and in paprika skin so that measurement can be easily reproduced. LÁNG (1972) worked with transmission spectra of different diffuse reflectance systems. Tomato juice and paprika

skin are also diffuse reflectance systems. Thus attempts were made to use *Láng*'s method. LÁNG and co-workers (1972) used painted paper and published methods to evaluate transmission spectra of paper sheets. They established that, in transmission spectra, maximum and minimum values characteristic of the pigment are much sharper than in reflection spectra. They suggested that neutral layers could sharpen maximum values by increasing diffusion. Any kind of spectrophotometer of proper sensitivity where the sample causing diffusion can be placed close enough to the light sensor of the spectrophotometer can be used for measurement (HUSZÁR, 1968; PÉTER, 1968).

We attempted to exploit results obtained in diffuse systems to determine maturity in tomato juice and paprika skin by colour measurement.

## 1. Materials and methods

Tomato samples were supplied by the RESEARCH INSTITUTE FOR VEGETABLE CULTIVATION, Kecskemét. These were partly of varieties in breeding, containing colour genes, thus, of high lycopene level, partly varieties in general cultivation. Tomato fruits were crushed cold and passed through a common household pulper of 0.4 mm mesh. Prior to measurements, the fibrous juice was de-aerated under vacuum. The de-aerated juice was used.

Paprika samples were supplied by the BREEDING STATION, Kalocsa of the RESEARCH INSTITUTE FOR VEGETABLE CULTIVATION.

To measure their spectrum, rectangular, level pieces of $1 \times 3$ cm were cut out from the paprika skin. After measurement, pieces were lyophilised to prevent pigment decomposition. The pigment content was determined by *Benedek*'s modified method.

Measurements were made with a *Specord* UV–VIS spectrophotometer, because in this instrument the samples can be placed close enough to the light sensor. It is highly important that diffusion in the cuvette in the path of the reference light and in the cuvette containing the sample to be measured should be the same. Diffusion of the cuvette containing the sample must be greater than that of the sample itself. To achieve this, the cuvettes were wrapped in filter paper of the type MN 640.

The tomato juice was poured into a cuvette and the transmission was measured. Prior to measurement the 0 point of the instrument was checked. Spectra were taken between 500 nm and 700 nm ($20 \cdot 10^{-3} - 14.285 \cdot 10^{-3} \nu$).

Stripes of paprika skin were placed into the cuvette prepared as above, on the side nearer to the light sensor.

Maturity of tomatoes which, in the present case of the juice, means its colour was measured with the *Momcolor* tristimulus colorimeter (HUNGARIAN OPTICAL WORKS, Budapest). The $X$, $Y$, $Z$ values were used to calculate $a_L/b_L$ ratios characteristic of colour.

## 2. Results

In the case of tomatoes and paprika, the fruits are ripe when the green colour disappears, *i. e.*, when the clorophyll level is reduced to zero and colour components characteristic of the ripe fruit develop. In tomato it is mainly lycopene, in paprika mostly capsanthin, and other red and yellow carotenoids. Accordingly, the method described is acceptable if the apparatus can sharply distinguish between the various stages of maturity.

Spectra measured as described above are shown in Fig. 1. The four spectra represent tomato juices at four different maturity stages. Spectrum No. 1 was obtained from unripe, green fruit; No. 4 from fully ripe fruit. Curve No. 1 has a characteristic maximum at 680 nm ($14.705 \cdot 10^{-3}\nu$) and a minimum between 500 ($20 \cdot 10^{-3}\nu$) and 605 nm ($16.528 \cdot 10^{-3}\nu$). The curve corresponds to the chlorophyll spectrum evaluated in organic solvents, naturally taking into account deviations caused by the solvents. It is interesting that the maximum is as sharp as in the case of organic solvents. As ripening progresses, more and more lycopene is found in the fruit; the minimum disappears, only a shoulder is left. The slope of curves corresponds to those of whole fruit taken by KRIVOSHIEV (1974). Figure 1 clearly proves that the maturity of tomatoes in juice form can be measured by the spectrophotometer.

Figure 2 shows the spectra of paprika stripes at different stages of maturity. The spectrum of green paprika is not shown, it would but unnecessarily complicate the figure. Spectra of red paprika stripes are similar to those of tomato juice. At the stage where both the red component (capsanthin) and the

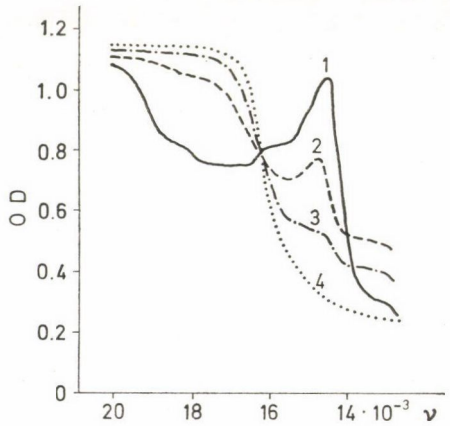


Fig. 1. Spectra of the four various ripened tomato's juice
——— 1: green tomato, unripe
— — — 2: pink tomato, half-ripened
—·—·— 3: red tomato, half-ripened
····· 4: red tomato, ripened

green component (chlorophyll) are present, there is a peak in the shoulder. At the stage of full maturity the peak disappears, only a shoulder is left. It is interesting that when the water content of the skin decreases (during after-ripening) the light transmission capacity increases. The shape of the curve indicates that it would suffice to observe the disappearance of the chlorophyll peak to determine maturity. In this case, however a recording spectrophotometer is absolutely needed.

In maturity determinations in tomato juice the task could be simplified by measuring light absorption at one wavelength only. But we must not forget that the solids content and with that the refraction of the juice at different maturity stages may widely vary and this affects light absorption. In the case of paprika skin, beside the colour components, the thickness of the skin also affects absorption. Skin thickness depends on variety, site of cultivation, moisture content. All these difficulties may result in incorrect measurements. We attempted to prevent difficulties by measuring absorption at two selected wavelengths instead of evaluating the whole spectrum. Since in measuring maturity no absolute absorption values are needed, with the ratio of absorption at two wavelengths we succeeded in eliminating error. Instruments constructed to measure maturity are all ratio meters. Measurements of light absorption at 588 nm ($17 \cdot 10^{-3} \nu$) and 680 nm ($14.705 \cdot 10^{-3} \nu$) was found to be suitable. These values are close to those of 575 nm ($17.391 \cdot 10^{-3} \nu$) and 675 nm ($14.814 \cdot 10^{-3} \nu$) of KRIVOSHIEV (1974).

Reproducibility determines whether a method may be used generally. Reproducibility values under the experimental conditions are shown in Fig. 3 separately for tomato and paprika. Reproducibility is lower by an order of magnitude than the usual error when using clear solutions. The coefficient of

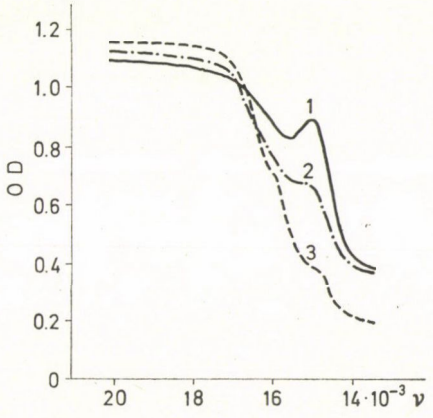


Fig. 2. Spectra of variously ripened paprika skin
——— 1: half red paprika skin
—·—·— 2: red paprika skin
— — — 3: red paprika skin after 40 days

| λ | $\bar{E}$ | s |
|---|---|---|
| 588 | 1.347 | 0.0105 |
| 680 | 0.509 | 0.0093 |
| $\frac{680}{588}$ | 3.481 | 0.0390 |

TOMATO JUICE

| λ | $\bar{E}$ | s |
|---|---|---|
| 588 | 1.237 | 0.0149 |
| 680 | 0.453 | 0.0123 |
| $\frac{680}{588}$ | 2.731 | 0.0520 |

PAPRIKA SKIN

Fig. 3. Reproducibility of the measures

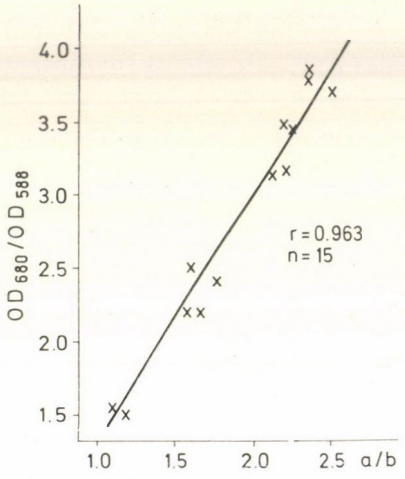


Fig. 4. Correlation between light absorbtion quotient with spectrophotometer and *Hunter* **L** *a/b* values in tomato juice

variation is, however, within 1%. The light absorption of the juice is above the ideal O. D. of the spectrophotometer (0.4–0.7) and this may account for the relatively high standard deviation. Reproducibility was determined by refilling the cuvettes prior to each measurement and paprika skins were removed and replaced again. Perhaps skins were not replaced properly into the cuvette, and this explains the high scatter. For tomato juice the spectrophotometric light absorption ratios were compared to *Hunter*'s *a/b* values. Comparison is shown

in Fig. 4. The agreement is good, as indicated by the high correlation coefficient, $r = 0.963$. Thus, it can be said that the spectrophotometer is a suitable means for determining maturity in tomato juice. The correlation between maturity and pigment level was calculated also for paprika skin and the coefficient was $r = 0.753$. Thus, the procedure is suitable for quality assessment according to maturity and conclusions may be drawn as to the pigment content.

We hope, we have succeeded in proving the adaptability of light absorption spectra to measure maturity, colour and pigment content in tomato and paprika.

The method enables delivery of the two produce according to quality.

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Addresses of the authors:

Dr. György Horváth — Institute of State Food Control and Chemistry, H-6001 Kecskemét, Széchenyi krt. 29., Hungary

László André — Institute of State Food Control and Chemistry, H-2892 Tata, Ady Endre út 29., Hungary

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# THE EFFECT OF PHYSICAL CHARACTERISTICS OF CIGARETTES ON THEIR BURNING PROPERTIES AND ON THE MAJOR COMPONENTS OF THE MAIN STREAM SMOKE

ARANY-FÜZESSÉRY, K., HAMZA-NAGY, I. and NAGY-BUDAY, I.

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The results of this study are related to data in the literature on the effect of the cut width, the diameter of cigarettes, the porosity (air permeability) of cigarette paper on the free burning of cigarettes and on the quantity of the major smoke components.

Mathematical statistical evaluations of the results permit the following conclusions:

- With increasing cut width, the mass and smoke yield of equally filled cigarettes decrease while the burning properties decline. An increase of cut width by 0.1 mm (within the range studied) reduces by 1–5% the amount of smoke components hazardous for health.
- The slightest change in cigarette diameter affects strongly the burning characteristics. A small reduction in diameter reduces the amount of the main stream smoke components.
- With an increased air permeability of the paper, the value of the burning characteristics increases. The extent of increase is linear up to a given porosity value independently of the composition of the product. Above this value the curves possess saturation character. Of the smoke components the total condensate and the quantitative change of alkaloids are exponentially related to porosity. The equations of the linearized correlations are acceptably applicable to the estimation of the effect of change in the porosity of paper on smoke composition.

The above results may greatly enable the improvement of the burning characteristics of cigarettes and the development of cigarettes of lower smoke yield.

The burning properties of cigarettes are important not only from the point of view of the sensory value but also from the health aspect. The free burning rate and the temperature of burning characterizing the burning properties affect substantially the amount of the biologically active components of smoke, its chemical composition.

The rate of free burning affects primarily the tobacco quantity burnt during smoking intervals and puffs and thus, the number of puffs and the smoke yield. The temperature of burning affects mainly the chemical composition of the main stream smoke.

The burning properties of cigarettes are decisively influenced by the tobacco used in them, but are affected also to a certain extent by the quality of the paper, the physical parameters of the product, the additives used in

manufacturing, the tobacco substitutes and last but not least the structure of cut.

In this study, the effects of some manufacturing characteristics on free burning, the composition of the main stream smoke and its quantity are accounted for. These are as follows:

- of the cut parameters cut width,
- of the cigarette parameters the diameter of cigarettes,
- the permeability to air of the paper.

It appears from data in the literature (SACHNOWSKY, 1965; FLESSELLES, 1967) that smoke yield decreases with increasing cut width while the burning properties slightly decline.

A generalizable quantitative comparison and an evaluation of the effect of the studied parameter are rendered more difficult by the circumstance that the parameters kept constant by various authors are different (SPEARS, 1974).

The influence of increase in the cigarette diameter on the burning properties is described by RESNIK (1974). He found that by increasing the diameter, at constant tobacco density, the burning rate of cigarettes decreases, while the burning index increases. In addition to the study of these parameters the change of the amount of smoke as a function of diameter within a given range was also investigated.

The effect of natural air permeability, that is, cigarette paper porosity was investigated by a number of authors (WYNDER & HOFFMANN, 1967; RICE *et al.*, 1970; RESNIK, 1974). It was found that the free burning characteristics change as a function of the porosity values of different cigarette papers and with increasing porosity the smoke yield decreases.

In the present study, the effect of air permeability was examined only in relation to the total condensate, the dry condensate and the alkaloid content of main stream smoke, because, based on data in the literature, substantial effects could be expected only in these fields. A further aim was to elucidate the mathematical correlations comprised on these changes.

## 1. Materials and methods

### *1.1. Test samples*

The test samples needed for the experiments were prepared at the pilot plant of the authors' institute on a *Dekajet* type cigarette manufacturing machine.

*1.1.1. Study of the effect of cut width.* Cuts of the nominal width of 0.5, 0.7, 0.9 and 1.2 mm were made of *Kállói B* strips tobacco variety, they were sufficiently homogenised and of appropriate fraction composition and cigarettes of the size of 80 × 8 mm, without filter, were prepared (Table 1).

Table 1

*Cut width of test samples*

| Sample | Nominal cut width (mm) | Cut width measured under the microscope (mm) | | |
|---|---|---|---|---|
| | | average | standard deviation | coefficient of variation (%) |
| 1 | 0.5 | 0.572 | 0.059 | 10.3 |
| 2 | 0.7 | 0.673 | 0.085 | 12.6 |
| 3 | 0.9 | 0.839 | 0.068 | 8.1 |
| 4 | 1.2 | 1.190 | 0.162 | 13.6 |

The tests were carried out with samples selected after conditioning, according to a given mass (average mass $\pm$ 20 mg) and filled identically. In case of wider cuts, it is possible to prepare identically filled cigarettes with lower filling mass.

*1.1.2. Examination of the effect of cigarette diameter.* The test samples were manufactured of the two fractions of *Kállói B* tobacco strips separated on tobacco screen. Fraction I contained strips above 25 $\times$ 25 mm, while fraction II those between 7 $\times$ 7 mm and 25 $\times$ 25 mm. Particles below 7 $\times$ 7 mm were not utilized. From fraction I, four (7.5, 7.6, 7.8, 8.1 mm) and from fraction II, three (7.6, 7.9, 8.1 mm) cigarettes of different diameter were made of 0.7 mm cut width and 80 mm length, without filter. The cigarettes were selected after conditioning according to their mass so that cigarettes made of the same fraction of different diameter shall be of the same density.

*1.1.3. Examination of the effect of cigarette paper porosity.* Test samples were produced from three different tobacco blends (marked S II, F and B) with papers of different porosity. The range of porosity was 13.2–185.5 cm $min^{-1}$ $kPa^{-1}$.

*1.2. Burning properties and smoke composition*

The burning characteristics and the smoke components were established by the routine methods used in this Institute (ARANY-FÜZESSÉRY *et al.*, 1977) after conditioning of the cigarettes during 48 h [$\varphi = 65\%$, $t = 293 \pm 2$ K (22 $\pm$ 2 °C)].

*1.2.1. The burning properties* may be characterized by the length of cigarette (burning rate) or by the mass of that burnt per minute without suction through it (burning index).

To measure the burning properties, use was made of the instrument developed at the Institute on the basis of the international standard (ISO 3612—1977), provided with 10 measuring places and automated time-keeper.

The burning time could be read on the time-keeper with 0.01 min accuracy. This value and the length (40 mm) or the mass of burnt cigarette were used to calculate the rate of burning or the burning index by the following formulae.

Burning rate

$$B_r = \frac{L_{40}}{T_{40}}$$

where $L_{40} = 40$ mm; $T_{40}$ = time in min required for the burning of 40 mm cigarette rod.

Burning index:

$$B_i = \frac{m_{40}}{T_{40}}$$

where $m_{40}$ = mass in mg of the cigarette rod of 40 mm.

*1.2.2. Analysis of smoke composition.* For these analyses, the cigarettes were smoked by an automatic smoking machine at parameters complying with the international (CORESTA STANDARD METHOD, 1968) and the HUNGARIAN STANDARD (1979): 35 $cm^3$ puffs of 2 s duration per min. The smoke was absorbed in different traps serving the purpose of analysis. The following smoke components were studied:

- crude or dry condensate,
- alkaloids,
- volatile phenols,
- oxo compounds,
- hydrogen cyanide.

To absorbe the above components the following traps and methods were used:

– the crude condensate was absorbed on glass fibre named *Cambridge* filter and it was determined by mass. The moisture content of the crude condensate was determined with *Karl-Fischer* reagent by titration.

– the alkaloids were obtained from the *Cambridge* filter by steam distillation and were measured from the distillate by UV spectrophotometry.

– the volatile phenols were collected on the *Cambridge* filter and obtained by steam distillation. The total phenol content of the distillate was determined according to *Folin-Ciocalteu*.

– the oxo compounds were trapped on silicagel. They were determined with an automatic colorimetric analyser, using dinitrophenyl hydrazine (DNFH) reagent, based on colour intensity measurements.

– the hydrogen cyanide was trapped in a gas-washing bottle in 2% NaOH solution and it was determined with ion-selective electrode by potentiometric titration.

## 2. Results and conclusions

### *2.1. Effect of cut width*

The results of the effect of cut width were summarized in Tables 2 and 3.

As can be seen, by increasing cut width, in cigarettes of identical filling (firmness):

- their mass decreases,
- their burning properties change unfavourably,
- the amount of the smoke components tested diminishes.

Table 2

*Effect of cut width on the physical properties and smoke components of cigarettes*

| Properties studied | Samples | | | |
|---|---|---|---|---|
| | 1 | 2 | 3 | 4 |
| Cut width (mm) | 0.572 | 0.673 | 0.839 | 1.190 |
| Mass of cigarette (mg) measured | 1 005 | 995 | 991 | 924 |
| Mass of cigarette (mg) calculated[a] | 991 | 972 | 971 | 924 |
| Burning rate (mm $min^{-1}$) | 3.28 | 3.27 | 3.11 | 3.04 |
| Burning index (mg $min^{-1}$) | 41.2 | 40.7 | 38.4 | 35.1 |
| Total condensate (mg per cigarette) | 42.5 | 39.9 | 39.6 | 38.8 |
| Alkaloids (mg per cigarette) | 2.39 | 2.29 | 2.18 | 1.94 |
| Volatile phenols (mg per cigarette) | 0.58 | 0.57 | 0.56 | 0.55 |
| Hydrogen cyanide (mg per cigarette) | 0.53 | 0.47 | 0.44 | 0.37 |
| Oxo compounds (mg per cigarette) | 1.23 | 1.13 | 1.16 | 1.10 |

[a] moisture content 13.0%, firmness 75%

Table 3

*Lowest significant differences belonging to the methods used as a function of parallel measurements*

| Properties studied | Number of parallel measurements | Significant difference |
|---|---|---|
| Burning rate (mm $min^{-1}$) | 10—10 | 0.15 |
| Burning index (mg $min^{-1}$) | 10—10 | 2.5 |
| Total condensate (mg per cigarette) | 10—10 | 2.13 |
| Alkaloids (mg per cigarette) | 5—5 | 0.12 |
| Volatile phenols (mg per cigarette) | 3—3 | 0.082 |
| Hydrogen cyanide (mg per cigarette) | 3—3 | 0.066 |
| Oxo compounds (mg per cigarette) | 3—3 | 0.143 |

Correlations were tested by one-way linear regression analysis. Results are given in Table 4. The same Table contains the relative changes belonging to $D_x = 0.1$ mm change in cut width. The relative effects thus obtained are in good agreement with data in the literature.

As can be seen from the correlation coefficients, a close correlation exists between cut width and the properties studied. Where the correlation coefficient lags only slightly behind the critical value, the equations describing the relations may be used for the estimation of tendency of the effect.

In summary, it was established that an increase of cut width by 0.1 mm reduces the amount of smoke components dangerous for health by 1–5%,

Table 4

*Regression correlations belonging to the physical and chemical properties as affected by cut width*

| Regression equations of the properties studied | Determination coefficient (%) | $r^a$ | Extent of change belonging to $D_x = 0.1$ mm |
|---|---|---|---|
| $Q = 1\,086 - 131\,x$ | 92.7 | 0.963 | −1.3 |
| $B_r = 3.51 - 0.42\,x$ | 90.2 | 0.949 | −1.3 |
| $B_i = 47.2 - 10.2\,x$ | 99.2 | 0.996 | −2.6 |
| $C = 43.92 - 4.59\,x$ | 73.3 | 0.856 | −1.1 |
| $A = 2.78 - 0.71\,x$ | 92.8 | 0.963 | −3.1 |
| $F = 0.60 - 0.39\,x$ | 55.6 | 0.750 | −0.7 |
| $HCN = 0.66 - 0.25\,x$ | 94.6 | 0.973 | −5.2 |
| $Oxo = 1.20 - 0.054\,x$ | 25.5 | 0.510 | |

Symbols: $x$ = cut width (mm)
$Q$ = mass of cigarette (mg)
$B_r$ = burning rate (mm min$^{-1}$)
$B_i$ = burning index (mg min$^{-1}$)
$C$ = total condensate (mg per cigarette)
$A$ = alkaloids (mg per cigarette)
$F$ = volatile phenols (mg per cigarette)
HCN = hydrogen cyanide (mg per cigarette)
Oxo = oxo compounds (mg per cigarette)

[a] critical value of the correlation coefficient, at df = 2; P = 95%; $r = 0.9500$

within the range studied. The increase of cut width is limited on the one hand by the decrease in burning properties and, by the irregularity of the filling of cigarettes on the other (WYNDER & HOFFMANN, 1967).

### *2.2. Effect of cigarette diameter*

Results obtained in relation to the influence of cigarette diameter are presented in Table 5.

The burning rate and the burning index show a linear correlation with the diameter. This means that even a slight change in diameter is followed by

Table 5

*Data of the burning properties of cigarettes of various diameter*

| Diameter (mm) | Number of fraction | Cut density (mg cm$^{-3}$) | Moisture content (%) | Burning index (mg min$^{-1}$) | Burning rate (mm min$^{-1}$) |
|---|---|---|---|---|---|
| 7.5 | I | 255.2 | 12.0 | 38.2 | 3.39 |
| 7.6 | | 256.5 | 11.7 | 39.0 | 3.35 |
| 7.8 | | 255.9 | 11.9 | 40.5 | 3.31 |
| 8.1 | | 256.4 | 11.9 | 42.8 | 3.24 |
| 7.6 | II | 261.5 | 11.8 | 41.4 | 3.49 |
| 7.9 | | 260.4 | 12.0 | 43.8 | 3.43 |
| 8.1 | | 261.0 | 11.8 | 45.1 | 3.35 |

a significant change in burning, however, in the opposing directions for the two characteristics. The linear regression equations describing the correlation and the parameters characterising their closeness are given as follows:

*Index of free burning*

Fraction I: $B_i = -19.085 + 7.64\,d$; $r \sim 1$; $r^2 = 99.9$

Fraction II: $B_i = -15.16 + 7.45\,d$; $r = 0.998$; $r^2 = 99.7$

where

$B_i$ = burning index (mg min$^{-1}$)

$d$ = diameter of the cigarette (mm)

*Rate of free burning*

Fraction I $B_r = 5.186 - 0.240\,d$; $r = 0.995$; $r^2 = 99.0$

Fraction II $B_r = 5.575 - 0.274\,d$; $r = 0.980$; $r^2 = 96.1$

where

$B_r$ = rate of burning (mm min$^{-1}$)

$d$ = diameter of the cigarettes (mm)

It appears from the slopes of the equations and from the diagrams in Figs. 1 and 2 that even a small change in diameter has a substantial effect on the burning index and burning rate.

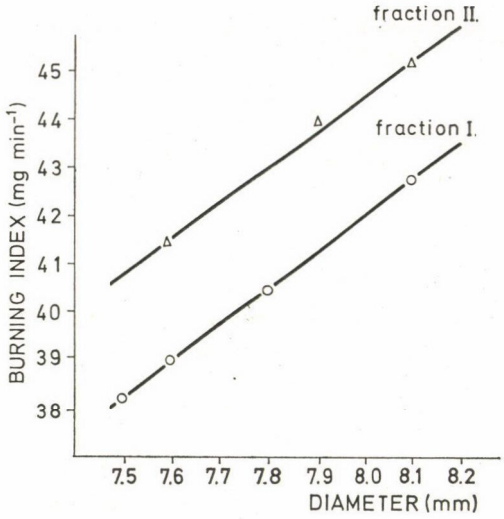


Fig. 1. Regression line representing the correlation between cigarette diameter and burning index
$-\bigcirc-\bigcirc$ Fraction I. ($B_i = -19.085+7.64\,d$; $r^2 = 99.9$)
$-\triangle-\triangle$ Fraction II. ($B_i = -15.160+7.45\,d$; $r^2 = 99.7$)

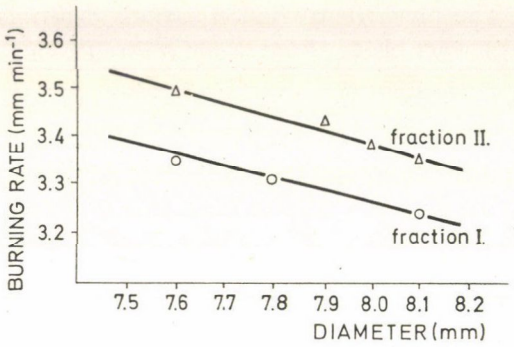


Fig. 2. Regression line representing the correlation between cigarette diameter and burning rate
$-\bigcirc-\bigcirc$ Fraction I. ($B_r = 5.186-0.240\,d$; $r^2 = 99.0$)
$-\triangle-\triangle$ Fraction II. ($B_r = 5.575-0.274\,d$; $r^2 = 96.1$)

The lines obtained for the two fractions (I, II) are practically parallel, *i.e.* the change in diameter within the studied range affects the burning characteristics independently of the fraction composition of the tobacco cut.

In the course of the analysis of the main stream smoke components, it was established that evaluations performed on a per cigarette or on a per tobacco mass basis lead to contradictory correlations. The amounts of smoke components calculated on a per cigarette basis generally show none or only slight increase with increasing diameter, while calculated on a tobacco mass basis, they diminish (Table 6).

Table 6

*Amount of main stream smoke components of cigarettes of varied diameter*

| Diameter (mm) | Fraction | Mass of cigarette (mg) | Total condensate | | Alkaloids | | Volatile phenols | | HCN | | Oxo compounds | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | (mg per cigarette) | (mg g$^{-1}$) | (mg per cigarette) | (mg g$^{-1}$) | ($\mu$g per cigarette) | ($\mu$g g$^{-1}$) | ($\mu$g per cigarette) | ($\mu$g g$^{-1}$) | (mg per cigarette) | (mg g$^{-1}$) |
| 7.6 | I | 932 | 40.1 | 43.0 | 2.05 | 2.20 | 806 | 865 | 394 | 423 | 1.35 | 1.44 |
| 7.8 | | 981 | 39.7 | 40.5 | 2.14 | 2.18 | 843 | 859 | 400 | 408 | 1.35 | 1.38 |
| 8.1 | | 1059 | 39.0 | 36.8 | 2.13 | 2.01 | 860 | 812 | 410 | 397 | 1.50 | 1.42 |
| 7.6 | II | 946 | 36.4 | 38.5 | 2.02 | 2.14 | 902 | 953 | 397 | 420 | 1.45 | 1.53 |
| 7.9 | | 1023 | 36.9 | 36.1 | 2.01 | 1.96 | 770 | 753 | 420 | 411 | 1.54 | 1.51 |
| 8.1 | | 1067 | 37.5 | 35.1 | 2.11 | 1.97 | 758 | 712 | 387 | 363 | 1.59 | 1.49 |

Finally, the results lead to the conclusion that a reduction in the diameter of the cigarette slightly reduces the amount of the main stream smoke components, if the reduction in diameter leads to a proportional reduction in tobacco cut.

*2.3. The effect of air permeability of the cigarette paper*

The effect of natural porosity on the burning index is shown in Fig. 3 and on the burning rate in Fig. 4.

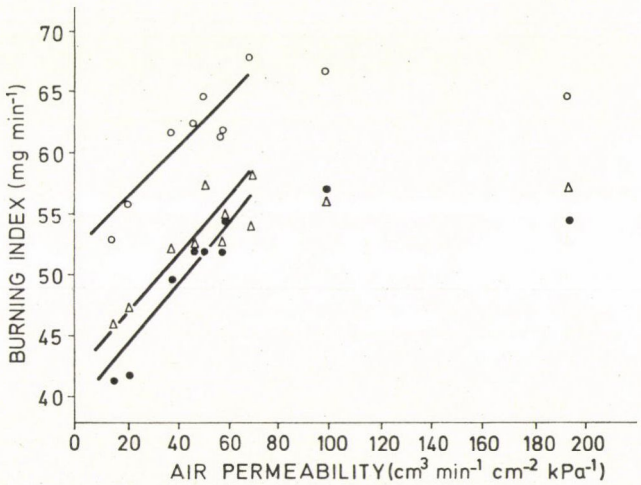


Fig. 3. Burning index as a function of the porosity of the cigarette paper (Test samples were produced from three different tobacco blends marked SII: —○—○—○; F: —△—△—; B: —.—.—)

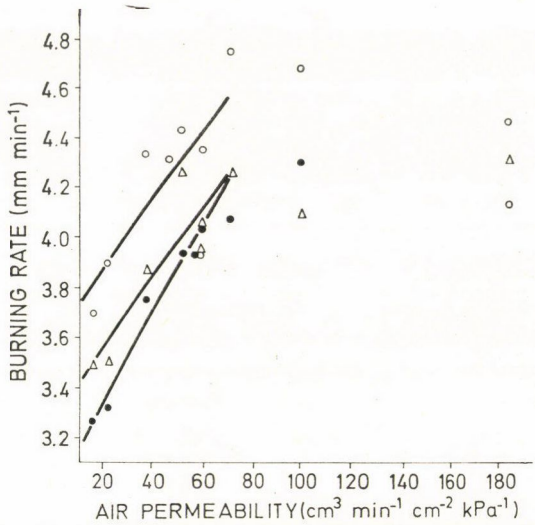


Fig. 4. Burning rate as a function of the porosity of the cigarette paper (Test samples were produced from three different tobacco blends marked SII: -○-○-○; F: —△—△—△—; B: —.—.—)

It can be seen that increasing porosity increases the value of burning characteristics. The increase seems to be linear up to a porosity of 70 cm $min^{-1} kPa^{-1}$, above this value the curves have a saturation character for everyone of the test samples. In the linear phase both the burning index and the burning rate substantially improve for all three mixtures (the curves are nearly parallel). This finding is supported by mathematical statistical calculations since the slopes do not differ statistically from one another. Thus, the generalized form of the correlations is given in the following equations:

$$\text{for the burning index: } B_i = a + 0.22\ \omega$$

$$\text{for the burning rate: } B_r = a + 0.015\ \omega$$

where

$$\omega = \text{air permeability } (\text{cm min}^{-1}\ \text{cm}^{-2}\ \text{kPa}^{-1})$$

The smoke yield is reduced by porous cigarette papers as a result of two effects. First, the free burning rate of cigarettes increases in puff intervals and thereby the number of puffs is reduced. Secondly, as an effect of the air infiltrating through the paper less tobacco is burnt by one puff.

The quantity of the smoke components studied as a function of porosity of the cigarette paper can not be approximated by linear equations but gives a curve of exponential character (Figs. 5 and 6).

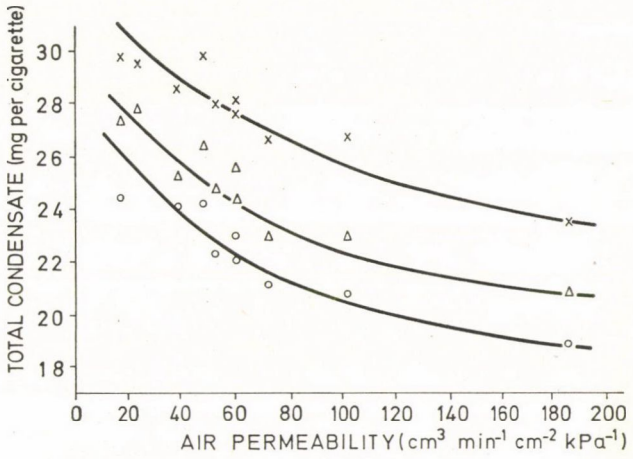


Fig. 5. Total condensate content of smoke as a function of the porosity of the cigarette paper
(Test samples were produced from three different tobacco blends marked SII: $-\circ-\circ-$; F: $-\triangle-\triangle-$; B: $-\times-\times-$)

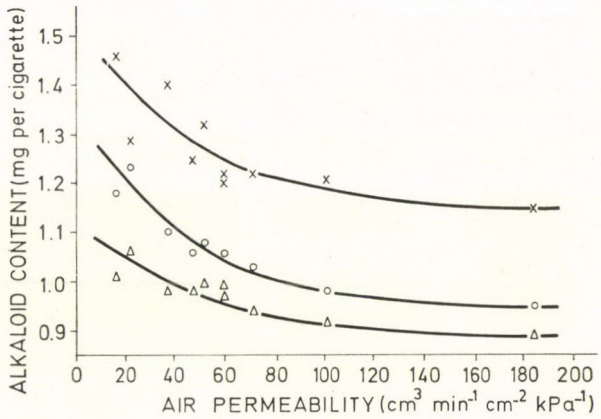


Fig. 6. Alkaloid content of smoke as a function of the porosity of the cigarette paper
(Test samples were produced from three different tobacco blends marked SII: $-\circ-\circ-$; F: $-\triangle-\triangle-$; B: $-\times-\times-$)

The curves were linearized by semi-logarithmic transformation and thus the closeness of the correlations could be calculated by linear regression. The characteristics of the correlations, the equations of the regression lines are summarized in Table 7.

The significance test of the slopes ($b$) of the lines obtained for the total condensate and for the alkaloids – at the probability level of 5% – shows that the slope of the lines does not differ. At the same time, the lines are independent of one another, because the intercepts ($a$) are different.

Table 7

*Analysis of the correlation between porosity of the cigarette paper and the total condensate and alkaloid contents, resp., in the main stream smoke*

| Tobacco used | Property studied | $r^a$ | $r^2$ | Equation of the regression line |
|---|---|---|---|---|
| S II | Total condensate | −0.890 | 0.792 | $y = 1.4137 - 0.0009\ x$ |
| | Alkaloids | −0.858 | 0.737 | $y' = 0.0681 - 0.0006\ x$ |
| F | Total condensate | −0.915 | 0.838 | $y = 1.4402 - 0.0007\ x$ |
| | Alkaloids | −0.740 | 0.548 | $y' = 0.0143 - 0.0004\ x$ |
| B | Total condensate | −0.989 | 0.978 | $y = 1.4849 - 0.0006\ x$ |
| | Alkaloids | −0.821 | 0.673 | $y' = 0.1397 - 0.0005\ x$ |

[a] Critical value of $r_{95\%} = 0.632$ (df = 8)
$x$ = Porosity of the cigarette paper (cm $min^{-1}$ $kPa^{-1}$)
$y$ = Logarithm of the total smoke condensate
$y'$ = Logarithm of the alkaloid content of smoke

Based on the above, the following equations of general validity may be formulated:

$$\text{for the total condensate: } y = a - 0.0007\ \omega$$

$$\text{for the alkaloid content: } y' = a' - 0.0005\ \omega$$

where

$y$ and $y'$ stand for the logarithm of the components investigated.

The equations may be used to establish the effect exerted by the porosity of the cigarette paper on the composition of smoke. This signifies an important advantage in quality assessment, in product development and in product up-dating and may replace time-consuming analyses.

This study on the effect of product characteristics is not considered completed. The study is continued on the effect of the characteristics on other smoke components hazardous for human health.

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Address of the authors:

Ms. Katalin ARANY–FÜZESSÉRY
Dr. Irén NAGY–BUDAY
Dr. Ilona HAMZA–NAGY

Tobacco Research Institute
H–4002 Debrecen, Attila tér 3.
Hungary

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# ENZYMATIC BROWNING, POLYPHENOL CONTENT, POLYPHENOL OXIDASE AND PEROXIDASE ACTIVITIES IN PEAR CULTIVARS

L. VÁMOS-VIGYÁZÓ and V. NÁDUDVARI-MÁRKUS

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The initial rate of browning (BA), the mass-related activities of polyphenol oxidase (PPO) and peroxidase (POD) as well as the o-dihydroxy phenol (ODP) and chlorogenic acid (CA) concentrations in mesocarp homogenates of five pear cultivars were determined. The enzyme activities were found to be unevenly distributed in the flesh of most cultivars. The degree of inhomogeneity was different in the cultivars, but similar with respect to the two enzymes. Of the characteristics studied, CA content showed the highest and mass-related PPO activity the slightest variation. CA content varied from 30 to 77% of o-dihydroxy phenol content. Close ($r^* = 0.866$) and significant ($P \leq 0.5\%$) linear correlation was found to exist between BA and the values of (ODP-CA), *i.e.* the part of the substrate consisting mainly of catechins. According to the results of multiple linear regression analysis, the variance of BA of the given population could be accounted for to 70% by the direct and joint actions of mass-related PPO activity and (ODP-CA) concentration. The influence of the latter variable on BA was about 4 times that of the former. The participation of mass-related PPO activity in the variance of BA could not be confirmed by statistical analysis.

Polyphenol oxidase (E. C. 1.14.18.1) (PPO) and peroxidase (POD) (E. C. 1.11.1.7) as present in plant tissues play an important role in fruit and vegetable processing and during storage of the processed goods. Within the scope of work aimed at elucidating the relative role of polyphenol oxidase and its endogenous substrates in the phenomenon of enzymatic browning, on the one hand, and to establish the true role of peroxidase in off-flavour development during storage of deep-frozen goods, on the other, the mass-related activities of these enzymes, the o-dihydroxy phenol (ODP) and chlorogenic acid (CA) contents and the initial rates of browning (BA) have been determined in several cultivars of different genera of fruits and vegetables such as apples, apricots, peaches, potatoes and carrots (GAJZÁGÓ *et al.*, 1977, 1979; SCHALLER & VÁMOS-VIGYÁZÓ, 1981; SCHALLER *et al.*, 1978, 1981; VÁMOS-VIGYÁZÓ & KISS-KUTZ, 1974; VÁMOS-VIGYÁZÓ *et al.*, 1976, 1977, 1980). The present paper deals with the results of investigations carried out into the above characteristics of 5 pear cultivars.

## 1. Materials and methods

### *1.1. The fruits*

The pears were harvested in 1976 at the Budakeszi model farm for educational purposes of the UNIVERSITY OF HORTICULTURE, Budapest. All cultivars were picked and analyzed at the state of canning maturity. The names of the

cultivars licensed for circulation since 1956 were as follows: 1. *Bosc kobak* (Boskoop), 2. *Hardenpont téli vajkörte* (Hardenpont Winter Butter pear), 3. *Diel vajkörte* (Diel Butter pear), 4. *Esperen bergamottja* (Bergamotte of S/N), 5. *Téli esperes* (Winter S/S).

*1.2. Determination of enzyme activities*

Spectrophotometric methods described earlier in this journal (MIHÁLYI & VÁMOS-VIGYÁZÓ, 1975, 1976) were used to determine the activities of PPO and POD in fruit homogenates. Homogenates (samples) were prepared in duplicate from 3 fruits each and 3 parallel spectrophotometric measurements were carried out for each activity. PPO activity was determined at pH 5.4 and 30 °C, using chlorogenic acid substrate. A change in optical density (OD) of $10^{-4}$ per min at 420 nm was adopted as unit activity. POD activity was assessed at pH 5.0 and 25 °C in reaction mixtures containing $H_2O_2$ and o-phenylene diamine as substrates. The activity unit adopted was $10^{-3}$ $\Delta$ OD per min as read at 420 nm. Both enzyme activities were related to 1 g of fruit-flesh (wet-mass basis).

*1.3. Determination of substrate concentration*

ODP-content was determined according to a modification of ALMÁSI and MOLNÁR's (1961) assay method based on the HOEPFNER (1932) reaction. The reaction mixture contained 1 $cm^3$ of fruit extract in methanol, 1 $cm^3$ of 0.5 *N* HCl, 1 $cm^3$ of $NaNO_2$-$Na_2MoO_4 \cdot 2H_2O$ solution (10% w/v each), 1 $cm^3$ of 1 *N* NaOH and 1 $cm^3$ of distilled water. The OD of the resulting coloured solution was read at 520 nm in a *Spectromom* 203, spectrophotometer (MAGYAR OPTIKAI MŰVEK, Budapest). ODP content was expressed as mg chlorogenic acid per 100 g of fruit flesh (wet-mass basis) using a calibration curve of chlorogenic acid treated in the above way.

CA-concentration was measured in the above fruit extract in methanol (prepared by homogenizing weighed aliquots of 3 pears and allowing the homogenate to stand overnight at 5 °C prior to centrifuging and filtration) by direct reading at 328 nm, the specific wavelength of absorbance of this compound (CÔME, 1971). Readings were related to a calibration curve obtained from a dilution series of chlorogenic acid in methanol and chlorogenic acid content was expressed in mg per 100 g of fruit flesh (wet-mass basis).

*1.4. Measurement of the initial rate of browning*

BA was measured on fruit slices in 6 replicates, by reflectance spectrophotometry at 540 nm, using the spectrocolorimeter *Spekol* (ZEISS, Jena) and was expressed in $\Delta R$ $min^{-1}$ ($\Delta R$ = change in reflectance in scale divisions; unit BA = 1 scale division per min, VÁMOS & GAJZÁGÓ, 1974).

The results were evaluated statistically by analysis of variance, after having tested the homogeneity of the variances by the *Bartlett*-test. Relation-

ships between the characteristics were established by simple and multiple linear regression analysis (SVÁB, 1973).

## 2. Results

### *2.1. Values of the characteristics studied*

The mean values of the mass-related PPO and POD activities, ODP and CA concentrations as well as BA values are represented in Fig. 1.

As can be seen, the pear cultivars investigated show considerable variations in all the characteristics studied. The heterogenity of the population is, however, different with respect to the individual characteristics. The ratios of the highest and lowest values for mass-related PPO and POD activities, ODP and CA contents as well as BA are, in the above order, as follows: 1.2, 2.5, 2.1, 3.9 and 2.2. The variations were slightest in mass-related PPO activities and highest in chlorogenic acid content.

According to the *Bartlett*-test, the variances proved to be homogeneous with respect to all the characteristics studied. The common $\chi^2$ values calculated

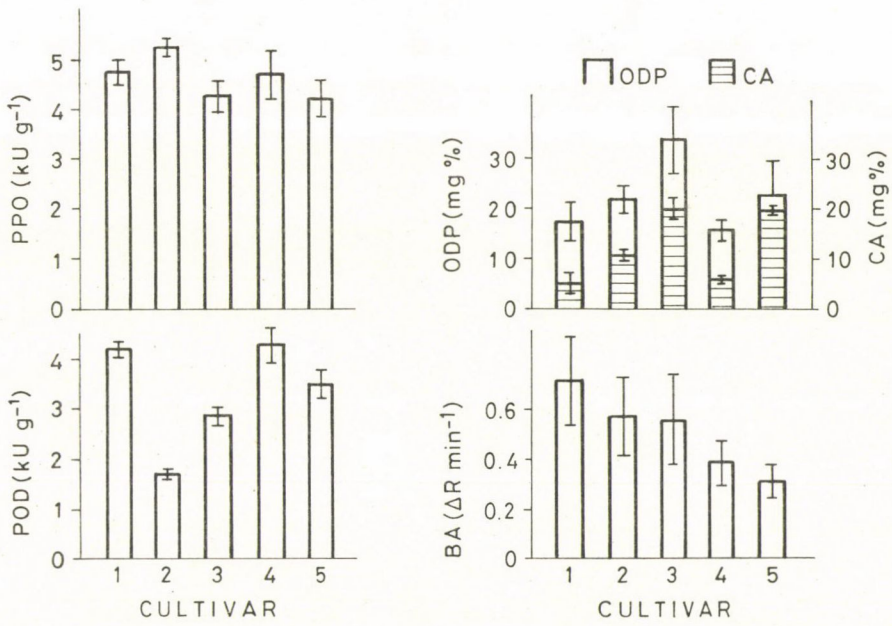


Fig. 1. Mass-related activities of polyphenol oxidase (PPO) and peroxidase (POD), o-dihydroxy phenol (ODP) and chlorogenic acid (CA) contents and initial rate of browning (BA) of five pear cultivars. Name of cultivars: 1. *Bosc kobak* (Boskoop), 2. *Hardenpont téli vajkörte* (Hardenpont Winter Butter pear), 3. *Diel vajkörte* (Diel Butter pear), 4. *Esperen bergamottja* (Bergamotte of S/N), 5. *Téli esperes* (Winter S/S). $R$ = reflectance. The vertical bars represent standard deviations

for the individual characteristics were, again in the above order: 3.81, 10.0, 3.7, 10.6, 11.9, while the table value for $\chi^2$ was 13.3 (df = 4; P = 1%).

The results of the comparison of the mean values of the characteristics studied as obtained by analysis of variance are given in Table 1 to 5.

Table 1

*Analysis of variance of the values of mass-related polyphenol oxidase (PPO) activity of five pear cultivars*

| *n* | *Source of variation* | *SS* | *df* | *MS* | *F* | $F_{table}$ | P % |
|---|---|---|---|---|---|---|---|
| 5 | Cultivars (c) | 4.28 | 4 | 1.07 | 12.77 | 7.08 | 1 |
| 2 | Samples (within cultivar) (s) | 1.80 | 5 | 0.36 | 20.00 | 10.48 | 0.1 |
| 3 | Replicates (within sample) (r) | 0.15 | 2 | 0.075 | | | |
| 4 | Total | 7.08 | 29 | — | | | |
| | Error (c) | 0.67 | 8 | 0.0838 | | | |
| | Error (c×s) | 0.18 | 10 | 0.018 | | | |

Least significant difference between cultivars: 0.39 (P = 5%); 0.56 (P = 1%); 0.84 (P = 0.1%)
Least significant difference between samples: 0.24 (P = 5%); 0.35 (P = 1%); 0.50 (P = 0.1%)

Multiple comparison of the mean values of mass-related PPO activity in the 5 cultivars

| Cultivar | *Boskoop* | *Hardenpont Winter Butter* | *Diel Butter* | *Bergamotte of S/N* |
|---|---|---|---|---|
| *Hardenpont Winter Butter* | 0.49* | — | — | — |
| *Diel Butter* | 0.50* | 0.99*** | — | — |
| *Bergamotte of S/N* | 0.04 | 0.53* | 0.46* | |
| *Winter S/S* | 0.54* | 1.03*** | 0.04 | 0.50* |

Comparison of the mean values of mass-related PPO activity in the samples (homogenates) within cultivar:

| Cultivar | Mean value (kU $g^{-1}$) | | Difference |
|---|---|---|---|
| | Sample A | Sample B | |
| *Boskoop* | 4.59 | 4.89 | 0.30* |
| *Hardenpont Winter Butter* | 5.37 | 5.10 | 0.27* |
| *Diel Butter* | 4.33 | 4.15 | 0.18 |
| *Bergamotte of S/N* | 4.25 | 5.14 | 0.98*** |
| *Winter S/S* | 4.44 | 3.96 | 0.48** |

*, **, ***: differences significant at the levels of P = 0.5%, 0.1% and 0.01%, resp.

Table 2

*Analysis of variance of the values of mass-related peroxidase (POD) activity of five pear cultivars*

| *n* | *Source of variation* | *SS* | *df* | *MS* | *F* | $F_{table}$ | $P_{\%}$ |
|---|---|---|---|---|---|---|---|
| 5 | Cultivars (c) | 26.98 | 4 | 6.75 | 232.76 | 14.39 | 0.1 |
| 2 | Samples (within cultivar) (s) | 0.56 | 5 | 0.112 | 4.48 | 3.33 | 5 |
| 3 | Replicates (within sample) (r) | 0.28 | 2 | 0.14 | | | |
| 30 | Total | 28.31 | 29 | — | | | |
| | Error (c) | 0.24 | 8 | 0.029 | | | |
| | Error (c×s) | 0.25 | 10 | 0.025 | | | |

Least significant difference between cultivars: 0.23 (P = 5%); 0.33 (P = 1%); 0.49 (P = 0.1%)
Least significant difference between samples: 0.29 (P = 5%); 0.41 (P = 1%); 0.60 (P = 0.1%)

Multiple comparison of the mean values of mass-related POD activity in the 5 cultivars:

| Cultivar | *Boskoop* | *Hardenpont Winter Butter* | *Diel Butter* | *Bergamotte of S/N* |
|---|---|---|---|---|
| *Hardenpont Winter Butter* | 2.48*** | — | — | — |
| *Diel Butter* | 1.34*** | 1.14*** | — | — |
| *Bergamotte of S/N* | 0.07 | 2.55*** | 1.41*** | |
| *Winter S/S* | 0.67*** | 1.81*** | 0.67*** | 0.74*** |

Comparison of the mean values of mass-related POD activity in the samples (homogenates) within cultivar:

| Cultivar | Mean value (kU $g^{-1}$) | | Difference |
|---|---|---|---|
| | Sample A | Sample B | |
| *Boskoop* | 4.14 | 4.23 | 0.09 |
| *Hardenpont Winter Butter* | 1.66 | 1.74 | 0.08 |
| *Diel Butter* | 2.95 | 2.74 | 0.21 |
| *Bergamotte of S/N* | 4.08 | 4.43 | 0.35* |
| *Winter S/S* | 3.28 | 3.72 | 0.44** |

For the explanation of the asterisks see *Table 1.*

The data of Table 1 and 2 show that significant differences in enzyme activities exist not only between cultivars, but also between the samples of a given cultivar. This indicates inhomogeneous distribution of the enzymes in the tissues. The multiple comparison of the mean values of the enzyme activities in the two samples of a cultivar permits conclusions as to the degree of inhomo-

Table 3

*Multiple comparison of the mean values of o-dihydroxy phenol concentration in five pear cultivars*

$[F = 5.84,\ F_{table} = 3.48\ (P = 5\%);\ \ (df_1 = 4;\ df_2 = 10)]$

| Cultivar | *Boskoop* | *Hardenpont Winter Butter* | *Diel Butter* | *Bergamotte of S/H* |
|---|---|---|---|---|
| *Hardenpont Winter Butter* | 4.2 | — | — | — |
| *Diel Butter* | 16.5*** | 12.3* | — | — |
| *Bergamotte of S/N* | 1.0 | 5.7 | 18.0** | |
| *Winter S/S* | 4.9 | 0.7 | 11.6* | 6.4 |

Least significant difference: 9.23 (P = 5%); 13.12 (P = 1%); 19.00 (P = 0.1%)
For the explanation of the asterisks see *Table 1.*

Table 4

*Multiple comparison of the mean values of chlorogenic acid concentrations in five pear cultivars*

$[F = 15.96\ ,F_{table} = 11.28\ (P = 0.1\%);\ \ (df_1 = 4;\ df_2 = 10)]$

| Cultivar | *Boskoop* | *Hardenpont Winter Butter* | *Diel Butter* | *Bergamotte of S/H* |
|---|---|---|---|---|
| *Hardenpont Winter Butter* | 5.4* | — | — | — |
| *Diel Butter* | 15.0*** | 9.4** | — | — |
| *Bergamotte of S/N* | 1.0 | 4.4 | 13.9*** | — |
| *Winter S/S* | 11.9*** | 6.5* | 3.0 | 10.9*** |

Least significant difference: 5.20 (P = 5%); 7.39 (P = 1%); 10.69 (P = 0.1%)
For the explanation of the asterisks see *Table 1.*

Table 5

*Multiple comparison of the mean values of the initial rate of browning of five pear cultivars*

$[F = 6.05,\ F_{table} = 4.18\ (P = 1\%);\ \ (df_1 = 4;\ df_2 = 25)]$

| Cultivar | *Boskoop* | *Hardenpont Winter Butter* | *Diel Butter* | *Bergamotte of S/N* |
|---|---|---|---|---|
| *Hardenpont Winter Butter* | 0.14 | — | — | — |
| *Diel Butter* | 0.15 | 0.01 | — | — |
| *Bergamotte of S/N* | 0.32** | 0.18 | 0.17 | — |
| *Winter S/S* | 0.39*** | 0.25** | 0.24* | 0.07 |

Least significant difference: 0.19 (P = 5%); 0.25 (P = 1%); **0.34** (P = 0.1%)
For the explanation of the asterisks see *Table 1.*

geneity of the samples. In the cultivar *Diel Butter* pear the distribution of both enzymes is to be considered homogeneous as the differences between the mean values of the samples are not significant. The distribution of both enzymes is the least homogeneous in the cultivars *Bergamotte of S/N* and *Winter S/S* the samples of which show the greatest significant differences. In the cultivars *Boskoop* and *Hardenpont Winter Butter* pear the samples proved to be inhomogeneous only with respect to PPO activity.

The comparison of the mean values of mass-related PPO and POD activities (Tables 1 and 2) shows significant differences between most of the cultivars. The cultivars *Boskoop* and *Bergamotte of S/N* do not differ significantly with respect to either mass-related enzyme activity and there is no significant difference in the mass-related PPO-activities of the cultivars *Diel Butter* and *Winter S/S*.

With respect to ODP-content only the cultivar *Diel Butter* (which showed the highest value) was significantly different from the rest (Table 3), while differences in CA concentration were significant between all but 3 pairs of cultivars *(Bergamotte of S/N* did not differ significantly from *Boskoop* and *Hardenpont Winter Butter*, neither did *Winter S/S* from *Diel Butter)* (Table 4). The ratio of CA/ODP varied over a wide range: from 0.30 *(Boskoop)* to 0.77 *(Winter S/S)*.

According to BA values, the population can be roughly divided into two groups: *Boskoop*, *Hardenpont Winter Butter* pear and *Diel Butter* pear have BA-values between 0.71 $\Delta R$ $min^{-1}$ and 0.56 $\Delta R$ $min^{-1}$ which do not differ significantly from each other, while the cultivars *Bergamotte of S/N* and *Winter S/S* have BA values 0.39 $\Delta R$ $min^{-1}$ and 0.32 $\Delta R$ $min^{-1}$, resp., which again do not differ significantly between each other (Table 5).

*2.2. Relationships between the characteristics studied*

At first sight no relationship seems to exist between the initial rate of browning and the mass-related PPO activity or ODP and CA concentrations, resp., of the five pear cultivars studied. However, when considering the difference of ODP and CA, *i.e.* the o-dihydroxy phenols beside chlorogenic acid, a distinct parallelism between these values and those of BA can be found. This can be expressed by a close linear correlation ($r^* = 0.865$), significant at the probability level of 95% ($P = 0.5$); $r^* = r\left(1 + \frac{1 - r^2}{2\,(n - 3)}\right)$ can be applied instead of $r = 0.792$ to indicate the significance of the correlation, because of the small number of data pairs (OLKIN & PRATT, 1958). The curve and the equation describing the correlation are given in Fig. 2.

As enzymatic browning necessarily depends on PPO activity as well, the interrelation of BA values, mass-related PPO activities and (ODP-CA) contents of the five pear cultivars were investigated by multiple linear regression

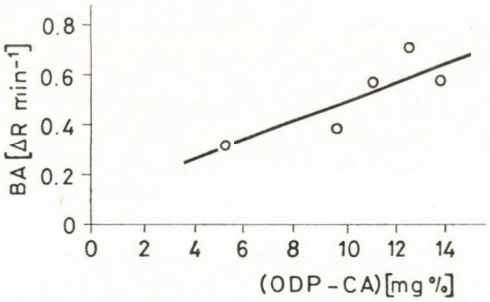


Fig. 2. Relationship between the initial rate of browning (BA) and the concentration of (o-dihydroxy phenols – chlorogenic acid) (ODP–CA) of five pear cultivars. $R$ = reflectance. The names of the cultivars are given in the legend to *Fig. 1.* Equation of the curve as obtained by linear regression: BA = 0.1088 + 0.03842 (ODP–CA)

analysis, the results of which are given in Table 6. The errors of the two independent variables (mass-related PPO-concentration and (ODP-CA) content) as well as of the dependent variable (BA) were disregarded in the calculations.

As can be seen, the linear correlation is significant at the probability level of 95%. The value of the multiple determination coefficient, $R^2$, indicates that about 70% of the variability of BA can be accounted for by the direct and joint effects of (ODP-CA) concentration and mass-related PPO-activity, whereby 59.57% and 3.82%, resp., of the variability are due to the direct effects of these two variables and 6.64% to their joint effect. Of the partial

Table 6

*Results of multiple correlation and linear regression analysis of the relationship between initial rate of browning (BA), o-dihydroxy phenol (ODP) content (without chlorogenic acid (CA) content) and mass-related polyphenol oxidase (PPO) activity of five pear cultivars*

| Cultivar | Sample | ODP-CA (mg %) | PPO-activity (kU $g^{-1}$) | BA ($\Delta$ R $min^{-1}$) |
|---|---|---|---|---|
| *Boskoop* | A | 12.27 | 4.59 | 0.71 |
| | B | 12.27 | 4.89 | 0.71 |
| *Hardenpont Winter Butter* | A | 11.07 | 5.37 | 0.57 |
| | B | 11.07 | 5.10 | 0.57 |
| *Diel Butter* | A | 13.87 | 4.33 | 0.56 |
| | B | 13.87 | 4.15 | 0.56 |
| *Bergamotte of S/N* | A | 9.74 | 4.25 | 0.39 |
| | B | 9.74 | 5.14 | 0.39 |
| *Winter S/S* | A | 5.27 | 4.44 | 0.32 |
| | B | 5.27 | 3.96 | 0.32 |

The regression equation: BA = −0.145 + 0.0365 (ODP-CA) + 0.0595 PPO
$r^2$ = 0.7026; $r$ = 0.8382

| *Variance factor* | *SS* | *df* | *MS* | *F* | Table value (P = 0.5%): |
|---|---|---|---|---|---|
| Total | 0.1900 | 9 | | | |
| Regression | 0.1335 | 2 | 0.06675 | 7.0885 | 4.74 |
| Error | 0.0565 | 7 | 0.00941 | | |

Significance of partial regression coefficients:

$b_1$ (ODP-CA) df = 7 $t = 3.6559$ $t_{table} = 3.50$ (P = 1%)
$b_2$ (PPO) df = 7 $t = 0.9260$ $t_{table} = 2.37$ (P = 5%)

Standardization of partial regression coefficients:

$b_1' = 0.7718$
$b_2' = 0.1954$

$\frac{b_1'}{b_2'} = 3.95$

Expansion of $r^2$:
(using the method of the path coefficients)

| | |
|---|---|
| Direct effect of (ODP-CA) | 59.57% |
| Direct effect of PPO: | 3.82% |
| Joint effects of (ODP-CA)×PPO: | 6.64% |
| | 70.03% |
| Other effects: | 29.74 |
| | 99.77% |

regression coefficients ($b_1$ and $b_2$) only that of (ODP-CA) ($b_1$) proved to be significant at the probability level of 99%. According to the ratio of the standardized regression coefficients the effect of (ODP-CA) on the initial browning rate of the five pear cultivars is about 4 times that of mass-related PPO activity.

## 3. Conclusions

### *3.1. The inhomogeneous distribution of enzyme activities in the tissues*

Both PPO and POD were shown, by analysis of variance of the data, to be unevenly distributed in the fruit tissues of most of the cultivars studied. A similar observation had been made earlier with peach cultivars (SCHALLER *et al.*, 1978). With peaches, this might be due to the fibrous structure of the mesocarp and with pears to the presence of grit cells. RANADIVE & HAARD (1972) found an elevated POD activity in the tissues surrounding the grit cells. The effect of the known inhomogeneity of enzyme distribution from the core towards the layers just below the skin were eliminated as much as possible by the mode of sampling, as samples were taken along the longitudinal axis of the fruit in the form of sectors including the mesocarp from the parts adjacent to the core (but without the core itself) to the layers just below the skin.

### *3.2. The values of the characteristics studied in the five pear cultivars*

From the values of the characteristics studied, those of o-dihydroxy phenol and chlorogenic acid contents can be compared to data of the literature. JURICS (1966, 1967) found in pears (cultivar not specified) 0.24 mg (+)-catechin and 0.71 mg (−)-epicatechin per 100 g of fruit flesh, while the chlorogen-

ic acid concentration was 25 mg per 100 g. In a more recent study MOSEL and HERRMANN (1974) reported on the presence, in 100 g fruit flesh of *Conference* and *Alexander Lucas* pears, of 0–0.6 mg of (+)-catechin, 0.7–12 mg of (−)-epicatechin and 9.6–21.6 mg of chlorogenic acid (determined, after hydrolysis, as caffeic acid). Catechins plus chlorogenic acid were found by MOSEL and HERRMANN (1974) to range from 10.6–10.8 mg % in *Conference* and 21.1–22.9 mg % in *Alexander Lucas* pears which latter contained also slight amounts of p-coumaric acid. This data fit in well with those established in the present study. The data cited show chlorogenic acid to be the dominating o-dihydroxy phenol of pears, constituting 90% or more of the phenolic substrates of enzymatic browning. In the present study such elevated ratios were not found and the great variations of the ratio within the population studied suggests a strong cultivar-dependence of the composition of phenolics in pears.

Concerning the other characteristics studied, it can be said that mass-related PPO and POD activities range with the bulk of the values found in apples and peaches, while BA is among the lowest found in apples, peaches and apricots (VÁMOS *et al.*, 1976, 1977; SCHALLER *et al.*, 1978) and ranges with the values found in the least browning of the apple cultivars studied, *i.e. Jonathan*.

### *3.3. Relationships between the characteristics studied*

The initial rate of browning of the five pear cultivars studied was foundto be primarily related to a special group of the endogenous substrates of PPO in the fruits, namely to those other than chlorogenic acid (mainly catechins). This is doubly interesting as *1)* in 3 out of the 5 cultivars tested chlorogenic acid made up 50% or more of the o-dihydroxy phenol content of the fruits and *2)* several authors (HALIM & MONTGOMERY, 1978; LUH *et al.*, 1963; RIVAS & WHITAKER, 1973; WALKER, 1964) found chlorogenic acid to be a far better substrate of pear PPO than (+)-catechin. However, it must be borne in mind that *a)* none of these authors tested (−)-catechin as substrate and (+)-catechin was found to be — as shown in section 3.2. — only a minor component of pear polyphenols not even present in all the lots; *b)* these authors used mainly *Bartlett* and *d'Anjou* pears for their studies and *c)* these studies were carried out with more or less purified PPO preparations, using the substrates separately and in the conditions of substrate saturation. Moreover, enzymatic browning might well be related to the concentration of catechins rather than to that of chlorogenic acid, as this latter compound yields products of a much fainter colouration when oxidized by PPO (MATHEIS & BELITZ, 1977; TÄUFEL & VOIGT, 1963).

The fact that the initial rate of enzymatic browning of the population of pears studied should primarily depend on (a group of the) endogenous polyphenols, is in good agreement with earlier findings and postulates of the authors: in experiments carried out during several years with 6 apple cultivars and

10 apricot cultivars (VÁMOS-VIGYÁZÓ *et al.*, 1976, 1977; GAJZÁGÓ *et al.*, 1977, 1979) it had been established that it was the ratio *(Q)* of mass-related enzyme activity and endogenous substrate content that determined which of these two factors of influence would play the limiting role in the enzymatic browning reaction (as characterized by the initial rate of browning). The higher the value of the above ratio, *i.e.* the lower the degree of relative substrate saturation, the greater the relative importance of endogenous substrate content in the browning reaction. For the population of pears considered in the present study, $Q$ values (kU $g^{-1}$ PPO activity per mg $g^{-1}$ ODP) ranged from 12.2 to 32, thus, according to the works cited, it was to be expected that substrate concentration should be the limiting factor of browning. It is, however, not clear why the correlation was obtained between BA and only a group of compounds of substrate. Experiments of the authors with 3 peach cultivars (SCHALLER *et al.*, 1981) had shown – in agreement with data of the literature (BUREAU *et al.*, 1977) – chlorogenic acid to be the limiting factor of the enzymatic browning reaction. It seems as if in both peaches and pears PPO had a preference for a given group of substrate compounds and, by the time these were depleted, reaction inactivation of the enzyme would be achieved, thus preventing the participation of other substrate compounds in the reaction. This assumption is, however, for the time being, merely speculative and requires further experiments for corroboration.

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Address of the authors:

DR. LILLY VÁMOS-VIGYÁZÓ
MS. VIKTÓRIA NÁDUDVARI-MÁRKUS

Central Food Research Institute
H-1525 Budapest, Herman Ottó út 15.
Hungary

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# CYTOKININ ACTIVITY OF *AGARICUS BISPORUS*

E. Kovács

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The cytokinin activity of *Agaricus bisporus* stored at a temperature of 15 °C and a relative humidity of 73–87% was determined for mushrooms which had reached various stages of cap opening. It was found that the cytokinin activity of the mushroom cap began to increase when the cap opening reached approx. 17–20%. This is equivalent to a width of 10 mm.

After purification and fractionation on a PVP poly(vinyl-pyrrolidone) column (pH 6.4 phosphate buffer), the cytokinin activity of mushrooms at different stages of opening was localised in fractions 7–9, 12–16 and 24–25 for the cap, 15–16 and 25–30 for the gills and 7–9 for the stem.

These purified cytokinin-active fractions stimulated the cap opening of freshly picked mushrooms but the growth of the stem was either inhibited or unaffected.

The active fractions could be identified by thin layer chromatography *Kieselgel* 60 $F_{254}$, (Merck, n-butanol – formic acid – water = 10:5:4) as zeatin and zeatin riboside.

The regulation of the metabolism of living organisms is one of the most urgent problems of modern biological research. Although excellent results have been achieved in numerous fields with organisms of a lower order, much less work has been done on problems connected with the regulation of metabolic processes in the cells of higher organisms, and a solution to these problems is not yet in sight. Mushrooms form a special field in this respect and deserve particular attention as important food substances of the future.

The results of previous research on mushrooms give good grounds for the assumption that the endogeneous hormone regulation systems of mushrooms and plants will be found to have a great deal in common. Thus, studies on the mechanisms behind these processes are particularly important, not only in order to clarify the theoretical basis of these processes, but also to discover new, efficient means of using them in agricultural practice. At present the auxins, gibberellins, cytokinins, and natural inhibitors such as ethylene and abscissic acid, are generally classified as phytohormones.

The *generic term cytokinins* is now used for numerous natural and synthetic substances, the vast majority of which have a *purine* skeleton, induce cell division and, in the presence of indol acetic acid (IAA), maintain the continuous growth of callus tissues.

These forms are transformed to a great extent in the course of the metabolism. The nucleotide forms of certain cytokinins are of common occurrence,

since they are incorporated into transfer-ribonuclein acid (tRNA) or bound to (rRNA,) and they play an important physiological role as nucleic acid components.

The structure of zeatin, the first natural cytokinin, was not identified until 1964 (LETHAM *et al.*, 1964). Consequently, the study of the effect of cytokinins on plant organisms is still only in the theoretical stage and it would be desirable to accelerate research on this subject.

MILLER (1967) isolated zeatin and zeatin riboside from the fruiting body of *Rhizopogon roseolus*, which forms a mycorrhizal association with the pine.

Investigations carried out by SZABÓ and co-workers (1970, 1972) and SZARVAS & POZSÁR (1977) showed that the cytokinin activity in fruiting bodies of *Coprinus micaceus* and *Agaricus bisporus* was nearly 500–600 mg $kg^{-1}$ (compared to the benzyladenine standard). The authors compared this cytokinin level to the endogenous cytokinin level of legumes and found that the cytokinin content of these mushrooms was 7–8 times greater thant the endogenous cytokinin level of the plants in question. It seems probable that the relatively intensive protein synthesis and nucleic acid metabolism of mushrooms is connected with their higher cytokinin content (POZSÁR, 1968). GOGOLA (1975) also demonstrated the cytokinin activity in *Agaricus bisporus* (using the tobacco callus assay; *Nicotiana tabacum* var. Wisconsin 38).

The only conclusion that can be drawn from research results on the cytokinin content of mushrooms is that these mushrooms do contain cytokinin, but its function has not yet been clarified.

Among the various works on growth substances in mushrooms, that of HAGIMOTO and KONISHI (1960) deserves special mention: when extracting the growth substances they used methods which resulted in the cytokinins being included in the extracts. At the time very little was known about the cytokinins, but it is very interesting to note that the properties of the growth substances found by these authors are also characteristic of the cytokinins.

## 1. Materials and methods

### *1.1. Experimental material*

For the experiments mushrooms with a cap diameter of 3–5 cm were used, since preliminary experiments showed the caps of these mushrooms to open at the same rate.

### *1.2. Radiation treatment*

Irradiation was carried out at the following places:

— At the INSTITUTE OF ISOTOPES OF THE HUNGARIAN ACADEMY OF SCIENCES (Budapest), with a $^{60}Co$ panoramic gamma ray source with a total activity of approx. 3.07 PBq.

— In the rest of the experiments a $^{60}Co$ panoramic gamma ray source with a total activity of 2.27 PBq, was used.

— The various solutions were irradiated with an RH-gamma-30 laboratory radiation source in the MICROBIOLOGICAL DEPARTMENT OF THE CENTRAL FOOD RESEARCH INSTITUTE, Budapest.

*1.3. Storage*

The samples were stored in uncovered boxes. The storage temperature was 15 °C, while the relative humidity varied between 73 and 78%. Any deviations from these conditions are noted for the individual experiments.

*1.4. Extraction of cytokinins*

The mushroom gills were homogenised with 80% ethyl alcohol (raw material–alcohol = 1:3) in a mixer. The mixture thus obtained was stored overnight in a refrigerator and then filtered. The pH of the filtrate was adjusted to 3 with 0.1 *N* HCl, then the majority of the impurities were removed by shaking the filtrate with four times its volume of ether. The pH of the aqueous phase containing cytokinin was adjusted to 6.5 with 0.1 *N* NaOH and the cytokinins were shaken into 4 volumes of n-butanol saturated with water. The butanol fraction was evaporated to dryness and, depending on the type of the experiment, was suspended in a definite volume of alcohol, n-butanol saturated with water or distilled water (LETHAM & WILLIAMS, 1969; KOVÁCS & VÖRÖS, 1975).

*1.5. Purification and separation of the extracts on an insoluble PVP column*

The large quantity of phenol type substances and other inhibitors present in champignons (WEAVER, 1969; WEAVER *et al.*, 1970; MADHOSINGHY, 1975) made it necessary to purify the samples. The method described below is suitable not only for the purification of the samples, but also for the separation of the cytokinins.

*Polyclar* AT (GAF GmbH, FRG) is an insoluble poly(vinyl-pyrrolidone) -copolymer (PVP) which forms a complex with phenols, so it can be used to remove the latter from plant extracts (ANDERSEN & SOWERS, 1968). A K-15 column (1.5 cm i.d., 30 cm length) was used for the analyses (PHARMACIA FINE CHEMICALS AG, Uppsala, Sweden) and the PVP was washed first with distilled water and then with phosphate buffer (pH 6.4). A 1.4 $cm^3$ sample was loaded onto the column, which was then eluted with pH 6.4 buffer at a rate of 30 $cm^3\ h^{-1}$. The optical density of the eluate at 254 nm was continuously measured (BIDDINGTON & THOMAS, 1973; 1976) with an LKB *Uvicord*.

*1.6. Thin-layer chromatography*

Thin-layer chromatography was used for the qualitative determination of the cytokinins. Preliminary experiments showed that a *Kieselgel* 60 $F_{254}$ (MERCK) layer was best suited for this purpose. The solvent system was in (upper phase) butanol – formic acid – water = 10:5:4 (LETHAM & WILLIAMS, 1969).

*1.7. Amaranthus betacyanin assay*

This biological test is based on the fact that numerous *Amaranthus* species synthesise red betacyanin in the cotyledon and the hypocotyl when exposed to light. In the dark the betacyanin synthesis is inhibited, but the synthesis can be induced in the dark in the presence of cytokinin and tyrosine.

*Amaranthus caudatus* L. seeds (SAMUEL DOBIE & SON LTD., England) were placed in a 31 × 16 cm plastic box containing two layers of *Whatman* 3 MM filter paper. The filter paper was moistened with distilled water and the seeds were germinated at 25 °C in the dark for 96 hours. The cotyledon and the upper 1 cm of the hypocotyl were used in the tests, which were carried out in round plastic boxes 6.5 cm in diameter, fitted with a lid. Two layers of *Whatman* 3 MM filter paper were put into each box and these were moistened with 3 $cm^3$ per box of the cytokinin-containing samples obtained in the course of fractionation. Three $cm^3$ phosphate buffer (pH 6.4) was put into the control boxes. Zeatin solutions with concentrations of 0.1, 0.01 and 0.001 ppm were used as the standards. Before testing, 2 $cm^3$ of a boiling solution of tyrosine with a concentration of 1 mg $cm^{-3}$ was added to each box. Ten seedlings per box were then placed into the nutrient solution and the samples were incubated in the dark at 25 °C for 18 h. After this the 10 seedlings were placed in a test-tube containing 3 $cm^3$ distilled water. Betacyanin was extracted by means of two cycles of freezing and thawing and the quantity determined by calculating the difference between the optical densities at 542 nm and 620 nm (BIDDINGTON & THOMAS, 1973).

*1.8. Effect of various mushroom extracts on the cap opening and stem growth of mushrooms*

The experiments were designed to determine what effect kinetin solutions of various concentrations have on the cap opening of mushrooms.

Freshly picked mushrooms with a closed cap 3–5 cm in diameter were used in the experiments. The hormone treatment was carried out in 20 × 25 × 4 cm porcelain bowls. Five hundred $cm^3$ solution were put into each bowl and covered with a sheet of plexiglass drilled with 15 holes, 2.5 cm in diameter each. The mushrooms were placed in the holes with their stems in the solution at a depth of 1 cm. The incubation time was 30–72 hours, the temperature 20 °C and the relative humidity 97–98%. The differences between the treatments were calculated with a portable *Hewlett-Packard* calculator using a $\chi^2$ test.

## 2. Results

### *2.1. Results of experiments on the cytokinin activity of Agaricus bisporus*

*2.1.1. Distribution of cytokinin activity in various parts of the mushroom.* The cytokinin activity in various parts of the mushrooms (cap without gills, stem and gills) showed the trends illustrated in Fig. 1.

In the cap, the greatest cytokinin activity was shown by fractions 7–9, 12–16 and 24–25. In the stem, fractions 7–9 were the most active, while in the gills fractions 15–16 and 25–30 showed cytokinin activity. Of the 30 fractions examined, fractions 7–8 were ninhydrin-positive. Since the most active fractions were found in the cap and the gills, these parts of the mushroom were studied in more detail. The activity of the stem was not studied separately, because the elution profils of the cap and the stem were similar.

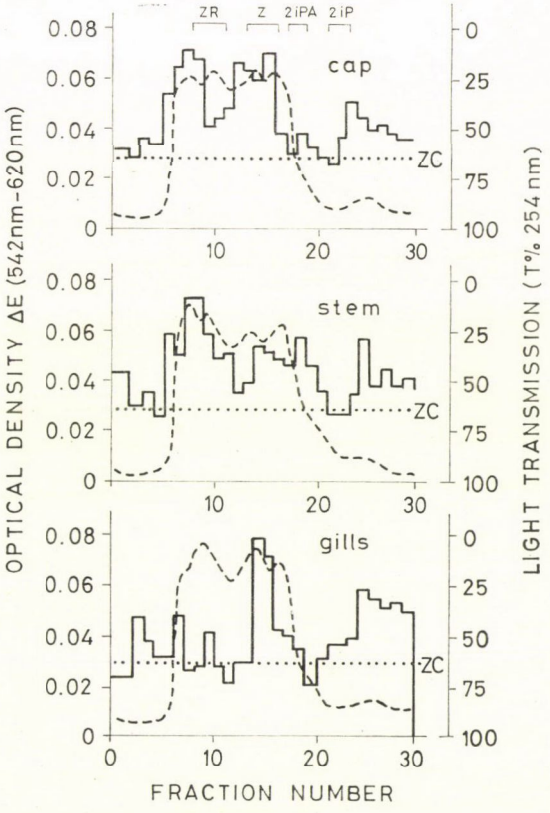


Fig. 1. Elution profile of extracts from various parts of *Agaricus bisporus* and the cytokinin activity of the fractions.
Fractionation in a K-15 (1.5 × 30 cm) column filled with insoluble PVP. Flow rate: 30 $cm^3$ $h^{-1}$. Eluent: phosphate buffer (pH 6.4). (· · · ZC = zeatin control, 0.1 mg $dm^{-3}$)
ZR = Zeatin ribosid; Z = Zeatin; iPA = Isopentyl adenosine; iP = Isopentyl adenine

Changes in the cytokinin activity of the cap and gills during storage were studied earlier (WAHID & KOVÁCS, 1980). In the latter study, it was established that the cytokinin activity in the cap showed a maximum value on the first day of storage and then decreased as a function of the storage time.

In the gills the activity was greatest on the first day after storage, then it showed a constant decrease as a function of the storage time. (The experimental results refer to mushrooms stored at a temperature of 15 °C and a relative humidity of 73–78%.)

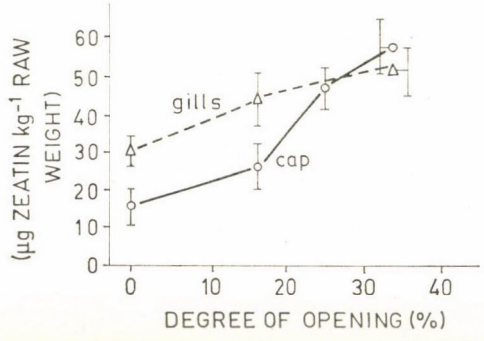


Fig. 2. Trend of cytokinin activity in mushrooms at different stages of cap opening (Temperature: 15 °C; Relative humidity: 73–78%)

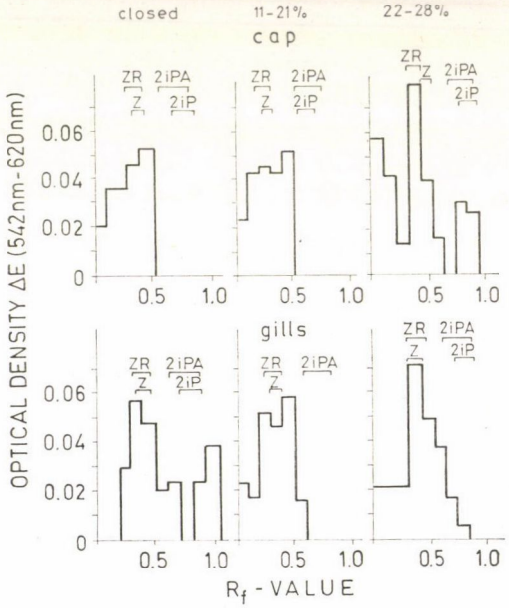


Fig. 3. Histograms showing the cytokinin activity of zones of chromatograms of samples derived from developing mushrooms (caps and gills).
*Kieselgel* 60 $F_{254}$ (MERCK); solvent system: n-butanol:formic acid:water = 10:5:4 (upper phase)

For abbreviations see Fig. 1

The rise in cytokinin activity in the cap seems to be correlated with several factors. Since a number of characteristic periods can be distinguished during the growth and development of the mushroom, it is conceivable that this too marks the beginning of a new period. The following experiment was designed to confirm this.

Freshly picked mushrooms with closed caps were stored, then after storage mushrooms with various degrees of cap opening were collected. The cap opening stages were selected on the basis of earlier morphological experiments. The storage conditions were the same as those in the previous experiment (temperature: 15 °C; relative humidity: 73–78%). The results indicate that the cytokinin activity of the cap begins to increase when the cap is approx. 20% open (Fig. 2). This stage of opening is equivalent to an opening width of approx. 10 mm and is reached when the velum breaks. The results show that the rise in activity occurs when the mushroom cap begins to open.

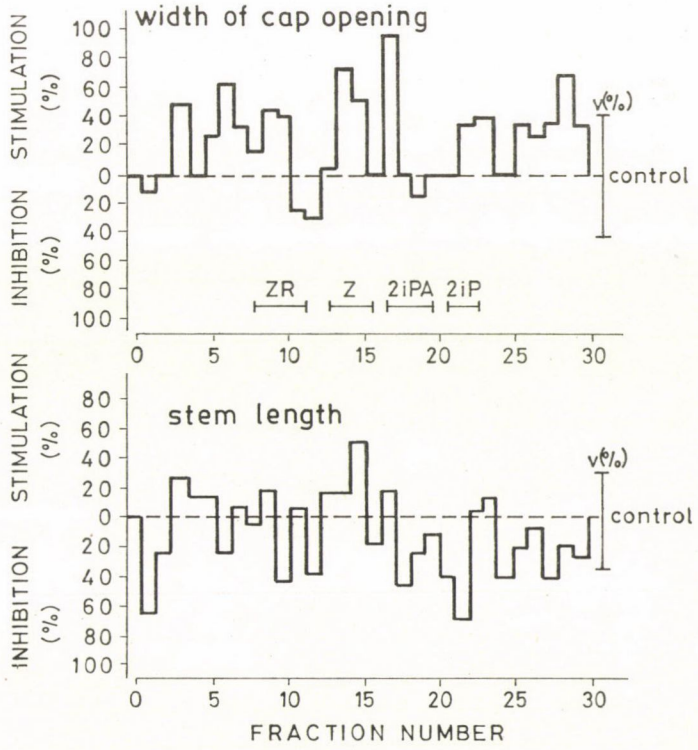


Fig. 4. Effect of fractions of purified extracts from the cap of *Agaricus bisporus* on the width of cap opening and stem growth in freshly picked mushrooms.
Incubation temperature: 20 °C; fractionation on a K-15 (1.5 × 30 cm) column filled with insoluble PVP. Flow rate: 30 $cm^3\ h^{-1}$; eluent: phosphate buffer (pH 6.4)
For abbreviations see Fig. 1

Considering that this is part of a complicated group of phenomena (cap opening, spore formation, cell growth and/or cell division), it is not yet possible to decide what is directly affected by the cytokinin.

The cytokinin extracts obtained from mushrooms in various stages of opening were separated by thin-layer chromatography and the cytokinin activity was illustrated as a function of the $R_f$ value. It can be seen that the most active substances coincide with the $R_f$ values of zeatin and zeatin riboside (Fig. 3).

*2.1.2. Effect of cytokinin-active substances on freshly picked* Agaricus bisporus. Fractions 14–16 of the cap extract, which exhibited cytokinin activity, proved to stimulate cap opening, resulting in the greatest increase in the degree of opening. These fractions did not have a significant effect on stem growth, while the other fractions tended to have an inhibitory effect (Fig. 4).

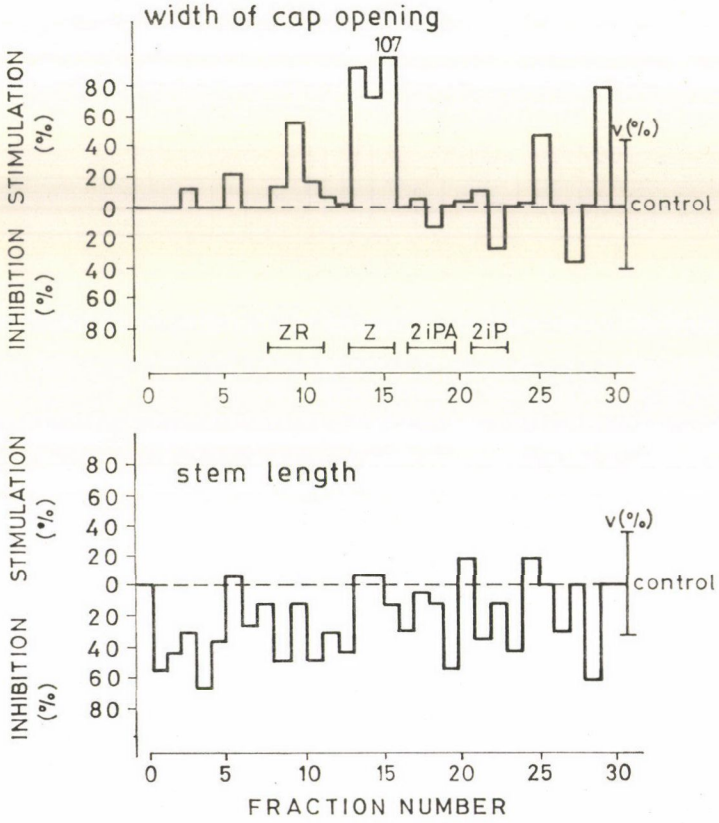


Fig. 5. Effect of fractions of purified extracts from the gills of *Agaricus bisporus* on the width of cap opening and stem growth in freshly picked mushrooms.
Incubation temperature 20 °C; fractionation on a K-15 (1.5 × 30 cm) column filled with insoluble PVP. Flow rate: 30 $cm^3\ h^{-1}$; eluent: phosphate buffer (pH 6.4)
For abbreviations see Fig. 1

Fractions 14–16 of the gill extract stimulated cap opening, but both these and the other fractions inhibited rather than stimulated stem growth (Fig. 5).

The fractions which had the greatest stimulating effect on cap opening were found at the elution site of zeatin. The figure also shows that more substances which stimulate cap opening are found in the mushroom cap than in the gills. However, it can also be seen that the growth of the stem is more strongly inhibited by various fractions of the gill extract than by the same fractions in the cap extract. The biologically active substances which have the greatest effect on cap opening generally have no effect on stem growth, or are possibly unable to manifest their effect due to the presence of other substances.

The results of previous studies on pileated mushrooms show that cytokinin-active substances are to be found in numerous species (CRAFTS & MILLER, 1974). Of these, cytokinin activity in *Agaricus bisporus* was demonstrated by POZSÁR (1968), SZABÓ and co-workers (1970; 1972), GOGOLA (1975), SZARVAS and POZSÁR (1977).

MILLER (1967) was the first of these authors to identify cytokinin in mycorrhizal fungi (*Rhizopogon roseolus*), and this proved to consist of zeatin and zeatin riboside, the most common of the natural cytokinins.

The present results show that there is a temporary increase in the cytokinin activity of the cap in *Agaricus bisporus*, coinciding with the beginning of cap opening.

Previous experiments showed that the number of spores also increased significantly when the cap began to open (KOVÁCS *et al.*, 1980). A similar temporary increase was observed in respiration by HAMMOND and NICHOLS (1975), and in respiration studies carried out by the authors.

It can be seen from the results that the various stages observed in the development of mushrooms when studying morphological changes (KOVÁCS & ZUKÁL, 1977) are in close correlation with the physiological processes in the mushroom. On the other hand, the most important change occurring during storage after the mushrooms are picked, *i.e.* the opening of the cap, is very closely correlated with the number of spores developing in the gills. These results are in good agreement with those obtained when studying the cytokinins and show that the cytokinins play an important role during storage after picking. It is not clear, as yet, whether they have a direct effect on cap opening or whether they promote cap opening by stimulating spore formation.

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Address of the author:

DR. ETELKA KOVÁCS Central Food Research Institute
H-1022 Budapest, Herman Ottó út 15.
Hungary

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# COMBINED EFFECT OF KINETIN AND RADIATION TREATMENT ON THE CAP OPENING OF *AGARICUS BISPORUS*

E. Kovács

(Received: 10 January 1981; accepted: 3 March 1981)

Cultivated mushrooms (*Agaricus bisporus*) with a cap diameter of 3–5 cm were used in the experiments. The mushrooms were incubated at 20 °C during the experimental period (30–72 h). The degree of cap opening was determined and the data were evaluated using a $\chi^2$ test.

It was found that kinetin solutions in the 0 to 32 mg $kg^{-1}$ range stimulated the opening of the mushroom cap. The shorter the time that elapsed between picking and treating the mushrooms, the greater was the effect.

A kinetin solution with a concentration of 100 mg $kg^{-1}$ inhibited the opening of the cap.

Cap opening in irradiated mushrooms cannot be induced even with kinetin concentrations that stimulate cap opening.

When kinetin solutions with a concentration of 100 mg $kg^{-1}$ were treated with 0, 2.5 and 10 kGy doses of radiation the stimulating effect on cap opening decreased as a function of the rising dose.

The radiation sensitivity of solutions of kinetin and various natural cytokinins (zeatin, 2iP and 2iPA) was studied and it was found that cytokinin solutions lost 50–60% of their activity after a radiation dose of only 1 kGy.

Cytokinins have been studied in numerous types of organisms, including not only plants and isolated plant parts, but also ferns, mosses, algae, mushrooms (Supniewski *et al.*, 1957), bacteria, viruses and even certain members of the animal kingdom (Kulaeva, 1973).

There are data to show that cytokinins stimulate the growth of mushrooms. This effect has been demonstrated on *Aspergillus niger* (Supniewski *et al.*, 1957) and also on other types of mushrooms (Skoog & Armstrong, 1970). In spite of all this, the subject has not been studied in sufficient depth and the available information is contradictory in nature. The data currently available are not sufficient to decide what correlation there is between this effect and the hormonal role of cytokinins in green plants.

## 1. Materials and methods

### *1.1. Experimental material*

Mushrooms obtained from the mushroom division of the Duna Cooperative Farm, Budapest were used in the experiments. Mushrooms with a cap diameter of 3–5 cm were chosen, as preliminary experiments showed that these mushrooms open at the same rate (Kovács *et al.*, 1968).

*1.2. Radiation treatment*

The radiation treatment was carried out using $^{60}Co$ gamma radiation sources with total activities of 3.07 and 2.27 PBq.

The irradiation of the various solutions was carried out using an *RH--gamma* 30 laboratory radiation source.

*1.3. Amaranthus betacyanin assay*

This bioassay is based on the fact that numerous *Amaranthus* species synthesise red betacyanin in the cotyledon and the hypocotyl when exposed to light. In the dark the betacyanin synthesis is inhibited, but the synthesis can be induced in the dark in the presence of cytokinin and tyrosine (BIGOT, 1968).

*Amaranthus caudatus* L seeds (SAMUEL DOBIE & SON Ltd., England) were placed in a $31 \times 16$ cm plastic box containing two layers of *Whatman* 3 MM filter paper. The filter paper was moistened with distilled water and the seeds were germinated at 25 °C in the dark for 96 h. The cotyledon and the upper 1 cm of the hypocotyl were used in the tests, which were carried out in round plastic boxes 6.5 cm in diameter, fitted with a lid. Two layers of *Whatman* 3 MM paper were put into each box and these were moistened with 3 $cm^3$ per box of the cytokinin-containing samples obtained in the course of fractionation, 3 $cm^3$ phosphate buffer (pH 6.4) was put into the control boxes. Zeatin solutions with concentrations of 0.1, 0.01 and 0.001 mg per kg were used as the standards. Before testing, 2 $cm^3$ of a boiling solution of tyrosine with a concentration of mg $cm^{-3}$ was added to each box. Ten seedlings per box were then placed into the nutrient solution and the samples were incubated in the dark at 25 °C for 18 hours. After this the 10 seedlings were placed in a test-tube containing 3 $cm^3$ distilled water. Betacyanin was extracted by means of two cycles of freezing and thawing and the quantity determined by calculating the difference between the optical densities at 542 nm and 620 nm (BIDDINGTON & THOMAS, 1973).

*1.4. Effect of kinetin on cap opening in* Agaricus bisporus

The experiments were designed to determine what effect kinetin solutions of various concentrations have on the cap opening of the mushrooms.

Freshly picked mushrooms with a closed cap of 3–5 cm in diameter were used in the experiments. The hormone treatment was carried out in $20 \times 25 \times 4$ cm porcelain bowls. Five hundred $cm^3$ solution were put into each bowl and covered with a sheet of plexiglass drilled with 15 holes, 2.5 cm in diameter each. The mushrooms were placed in the holes with their stems in the solution at a depth of 1 cm. The incubation time was 30–72 h, the temperature 20 °C and the relative humidity 97–98%. The differences between the treatments were calculated with a portable *Hewlett-Packard* calculator using a $\chi^2$ test.

## 2. Results

### *2.1. Effect of kinetin solutions of various concentrations*

The effect of kinetin solutions with concentrations in the range of 0 to 32 ppm (0, 2, 4, 8, 10 and 32 mg $kg^{-1}$) on the cap opening in champignon mushrooms is shown in Fig. 1. It can be seen in the figure that the cap opening of mushrooms with closed caps, *i. e.* those treated immediately after picking, can

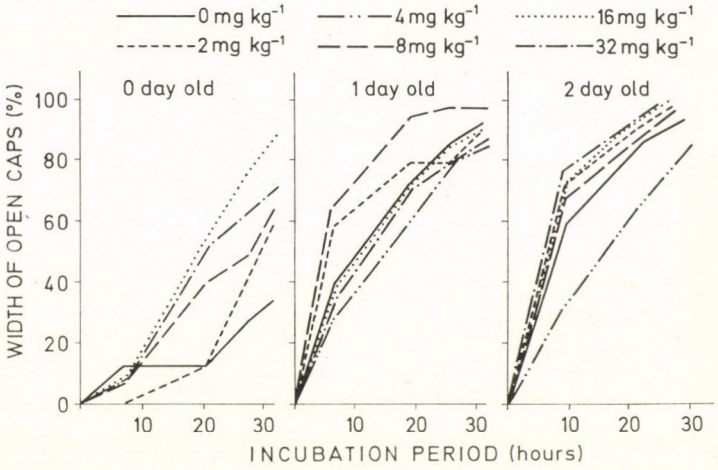


Fig. 1. Effect of kinetin solutions with various concentrations on cap opening in *Agaricu bisporus* (Incubation temperature 20 °C)

Table 1

*Effect of 0, 4, 8, 16 and 32 ppm kinetin solutions on the cap opening of freshly picked mushrooms based on the frequency distribution of widths of cap opening*

| Storage time (days) | n | Incubation time (hours) at 20 °C temperature | | | | | |
|---|---|---|---|---|---|---|---|
| | | 17—19 | | 21.5—25.5 | | 25.5—31 | |
| | | *df* | $\chi^2$ value | *df* | $\chi^2$ value | *df* | $\chi^2$ value |
| 0 | 360 | 20 | 36.41* | 20 | 41.57** | 25 | 56.86*** |
| 1 | 255 | 20 | 28.82 | | | 20 | 56.73*** |
| 2 | 294 | 20 | 20.22 | | | 20 | 20.48 |

n = number of individuals examined (data from 3 experiments are evaluated together, as the opening of the control mushrooms showed identical frequency distribution in the 3 mushroom populations)
df = degrees of freedom

* = significant at $P \geq 95\%$ probability level
** = highly significant at $P \geq 99\%$ probability level
*** = very highly significant at $P \geq 99.9$ probability level

be stimulated with these concentrations of kinetin. Cap opening was stimulated most strongly by 16 and 32 mg $kg^{-1}$ solutions. The stepwise cap opening of untreated mushrooms, which is well illustrated in the figure, gradually disappears as the kinetin concentration increases.

The stimulating effect of kinetin on cap opening is still exhibited in mushrooms stored for 1 day, though the effect is less strong than with freshly picked mushrooms. The statistical data in connection with this figure are presented in Table 1. The kinetin treatment was only effective for freshly picked mushrooms (0 day old) or for those stored for 1 day after picking.

The data grouped according to the different stages of opening determined in the preliminary experiments and the statistical evaluation of these data is presented in Table 2.

After 25.5–31 h of storage the number of mushrooms with closed caps differed from the theoretical mean as the results of the treatments in the following cases: for 4, 8 and 16 mg per kg concentrations the number of mushrooms with closed caps was 17, 8 and 7, *resp.*, less than the theoretical mean, *i.e.* these mushrooms opened more intensively. This is also shown by the fact that more mushrooms are found in the groups with wider cap openings, as shown by numbers prefixed with a + sign, than were expected from the theoretical calculations.

Mushrooms treated with 100 ppm kinetin solution opened to a lesser extent and the number of mushrooms with closed caps was higher than the theoretical mean. A comparison of the frequency distribution for all the treatments and for mushrooms at all stages of cap opening at each period of time was carried out by means of a multifactorial $\chi^2$ test. It can be seen from the table that there is a significant difference in the effects of various treatments. (Within the $\chi^2$ values for the groups examined, the effect of individual treatment is shown in the table by numbers prefixed with + and — signs. These numbers indicate significant deviations in a positive or negative direction from the mathematically calculated theoretical mean.)

### *2.2. Effect of kinetin solutions with various concentrations on irradiated* Agaricus bisporus

The results of previous examinations showed that radiation treatment inhibits the processes which are responsible for cap opening (KOVÁCS *et al.*, 1968) and spore formation (KOVÁCS *et al.*, 1981) in the mushrooms. It was found that, depending on the concentration, kinetin solutions either stimulate or inhibit cap opening. The following experiments were aimed at determining whether the effect of radiation treatment could be cancelled by those concentrations of kinetin which stimulate cap opening. The statistical data presented in Table 3 show that the effect of irradiation cannot be cancelled by these

Table 2

*Effect of kinetin solutions of various concentrations on the cap opening of freshly picked mushrooms*

| Treatments (mg kg$^{-1}$) | n | Incubation time (hours) at 20 °C temperature | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | 17—19 | | | | 21.5—25.5 | | | | | 25.5—31 | | | | |
| | | degree of opening (%) | | | | | | | | | | | | | |
| | | 0 | 1—10 | 11—21 | 22—28 | 0 | 1—10 | 11—21 | 22—28 | 29—38 | 0 | 1—10 | 11—21 | 22—28 | 29—38 |
| Control | 30 | | +3 | | −3 | | | | | | | | | | |
| 0.5 | 30 | | | | | | | | | | | | | | |
| 2 | 30 | | | | | | | | | | | | −2 | +5 | −3 |
| 4 | 30 | −18 | | +9 | +7 | −19 | | | +9 | +8 | −17 | | | | +17 |
| 8 | 30 | | −2 | | +2 | −7 | | | +4 | +3 | −8 | | | +3 | +5 |
| 16 | 30 | | | −3 | +2 | | | | | | −7 | | | +3 | +3 |
| 100 | 30 | +7 | −2 | −4 | −1 | +8 | | −3 | −3 | | +7 | −2 | | | −4 |
| | | $df$: $(7-1)\cdot(4-1)=18$ | | | | $df$: $(7-1)\cdot(5-1)=24$ | | | | | $df$: $(7-1)\cdot(5-1)=24$ | | | | |
| | | $\chi^2 = 48.90^{**}$ | | | | $\chi^2 = 54.75^{***}$ | | | | | $\chi^2 = 63.14^{***}$ | | | | |

Note: The frequency distributions of all the treatments were compared using a multifactorial $\chi^2$ test
The numbers in the table indicate the deviation in a positive (+) or negative (−) direction from the theoretical mean
$df$ (degrees of freedom) = (number of treatments − 1) · (number of groups for degree of opening − 1)

n = number of mushrooms examined
** = highly significant at P $\geq$ 99% probability level
*** = very highly significant at P $\geq$ 99.9% probability level

concentrations of kinetin, either in freshly irradiated mushrooms or when treated with kinetin 1 or 2 days after irradiation, as there is no significant difference between the effects of different concentrations of kinetin. The caps of irradiated mushrooms did not open as the result of kinetin treatment.

Table 3

*Test of significance on the effect of treatment with kinetin solution on mushrooms irradiated with 2.5 kGy*

Kinetin concentrations: 0, 1, 2, 4, 8 and 16 ppm

| Storage time (days) | Experiment | n | Incubation time (hours) at 20°C temperature | | | |
|---|---|---|---|---|---|---|
| | | | 20—22 | | 40—43 | |
| | | | *df* | $\chi^2$ | *df* | $\chi^2$ |
| 0 | 1 | 30 | 16 | 24.95 | 24 | 36.25 |
| | 2 | 30 | 12 | 4.61 | 24 | 22.59 |
| | 3 | 30 | 12 | 22.38 | 24 | 40.64 |
| 1 | 1 | 30 | 15 | 18.30 | 25 | 14.52 |
| | 2 | 30 | 18 | 24.13 | 24 | 14.77 |
| | 3 | 30 | 10 | 22.47 | 18 | 34.47 |
| 2 | 1 | 30 | 16 | 27.63 | 24 | 38.53 |
| | 2 | 30 | 15 | 24.73 | 24 | 26.36 |
| | 3 | 30 | 16 | 21.41 | 24 | 35.42 |
| 3 | 1 | 30 | 14 | 10.03 | 24 | 20.44 |
| | 2 | | | | | |
| | 3 | 30 | 16 | 21.41 | 16 | 21.45 |

n = number of mushrooms examined
df = degrees of freedom
Note: Each $\chi^2$ value was obtained by comparing 6 treatments. Each experiment was carried out in 3 replications. The effect of treatment with kinetin solutions was not significant in any case

### *2.3. Examination of the radiation sensitivity of various cytokinin solutions*

Solutions of kinetin, zeatin, 2iP [6($\gamma$, $\gamma$-dimethyl-allyl-amino)-purine] and 2iPA [6($\gamma$, $\gamma$-dimethyl-allyl-amino)-purine riboside] (CALBIOCHEM) were irradiated with doses of 1, 2 and 5 kGy, then the activity of the solutions was determined using the *Amaranthus* betacyanin assay (Fig. 2).

The results show that the cytokinin solutions tested lose 50–60% of their activity after a radiation dose of only 1 kGy. The activity of the cytokinin solutions decreased significantly as a function of the rise in radiation dose.

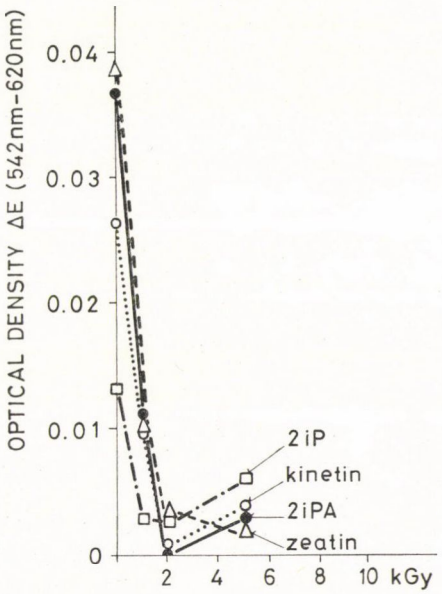


Fig. 2. Examination of radiation sensitivity of various cytokinin solutions using the biological *Amaranthus* betacyanin assay

## 3. Conclusions

Many attempts have been made to stimulate plant growth with cytokinins, but so far no efficient method has been found for improving the growth and yield of plants. Consequently, the relevant results show considerable variation. The growth of tomato, cucumber and pea can be inhibited and flowering delayed by adding kinetin solutions with a concentration of 0.02–2.0 mg $kg^{-1}$ to the nutrient medium. Other data show that the growth of apples can be promoted by cytokinin treatment (LETHAM, 1969). A single treatment with a highly concentrated kinetin solution, combined with a satisfactory source of nitrogen, resulted in a significant increase in the total weight of sunflower plants and an increase in the weight of the inflorescence (KULAEVA, 1973).

Experiments carried out by the author show that cap opening in mushrooms can be stimulated by kinetin solutions with concentrations of 0–32 mg $kg^{-1}$, while 100 mg $kg^{-1}$ solutions act as growth inhibitors. This latter effect can be utilised to develop a method for improving the storability of mushrooms. SZABÓ and co-workers (1972) found that 1–10 mg $kg^{-1}$ solutions of kinetin and benzyladenine stimulate the growth and dry matter content of the mycelium in *Agaricus bisporus*, while these effects are inhibited by 100 mg $kg^{-1}$ solutions.

The results of the radiation treatments indicate that the opening of irradiated mushroom caps cannot be induced by cytokinin solutions (Table 3). This means that the mechanism through which the cytokinins act has been damaged. At the same time, it can be seen that the activity of kinetin solutions decreases if they are treated with doses of 1 kGy or more (Figs. 2 and 3).

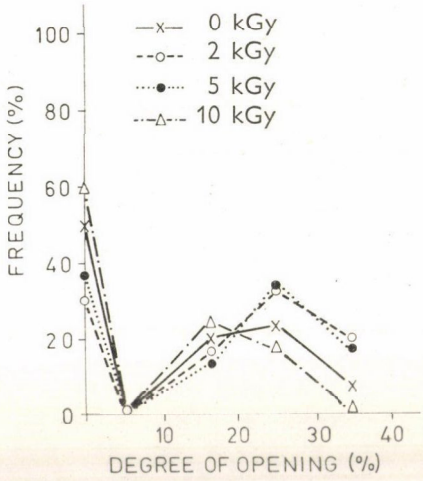


Fig. 3. Frequency distribution of opening widths after treatment with 10 mg $kg^{-1}$ kinetin solutions irradiated with 0, 2, 5 and 10 kGy
(Incubation temperature 20 °C; n = 30; significant at $P \geq 95\%$ probability level)

Unpurified gill extract stimulated cap opening even after treatment with 2, 5 or 10 kGy (TÖRLEY, 1970). There may be several reasons for this. Firstly, mention should be made of the protective effect of the extract; furthermore, it is not known what other substances were present in the extract, and naturally the stimulating effect on opening exhibited by the mushroom itself cannot be ignored either.

JORDAN and HABER (1974) showed that there was a decrease in cytokinin activity following irradiation in the seedlings of cereal seeds treated with 5 kGy.

Considerable cytokinin activity was displayed by *Haworthia* callus after treatment with 0.1–0.2 kGy, although no activity could be demonstrated in non-treated samples (PANDEY *et al.*, 1978).

On irradiating tomatoes (San Marzano) with 0.4–1.6 kGy it was found that if the tomatoes were treated before they were completely ripe, the cytokinin activity of irradiated samples was maintained better during storage (KOVÁCS & VÖRÖS, 1975).

These results show that ionising radiation may induce or inhibit cytokinins, depending on the radiation dose and on the state of maturity of the treated product. This fact widens the scope for using ionising radiation in agriculture.

*

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Address of the author:

DR. ETELKA KOVÁCS
Central Food Research Institute
H-1022 Budapest, Herman Ottó út 15.
Hungary

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# INCORPORATION OF L-METHIONINE INTO CASEIN HYDROLYSATE BY ENZYMATIC TREATMENT

GY. HAJÓS and A. HALÁSZ

(Received: 17 January 1981; accepted: 6 March 1981)

Enzymatic resynthesis, the plastein reaction, allows incorporation of limiting essential amino acids into proteins, thereby improving the nutritional quality of the latter. In our laboratory, L–methionine methyl ester was incorporated into the appropriate hydrolysate of casein ($\alpha_H = 85\%$) by the plastein reaction. It was assumed that, when L-methionine methyl ester is incoroprated into the protein chain by covalent bonds, an equivalent amount of methanol is formed. The methanol content in the supernatant of the plastein reaction samples was extracted by steam distillation and determined by gas chromatography. Studies on the precipitates formed in the enzymatic resynthesis were carried out after dialysis. Amino acid content and changes in the methionine proportion were controlled by thin-layer ion-exchange chromatography as well as by amino acid analysis. A significant increase – an approximately 4-fold enrichment – in the methionine content of plasteins was found due to the incorporation of L-methionine methyl ester during the enzymatic process. Our experimental results are in agreement with a covalent linkage of methionine to the plasteins.

The methionine proportion in proteins of non-animal origin applied in the food industry is generally surprisingly low. Enrichment in form of free amino acids is, however, not a suitable way to supplement essential amino acids (ALTSCHUL & HARON, 1974). Therefore, the increase in the content of methionine as one of the most important limiting essential amino acids would be very important in the practice of the food industry. For this reason, experiments have been made in our laboratory for incorporation of methionine by enzymatic processes.

Enzymatic protein degradation and the resynthesis processes, the so called plastein reaction is a suitable method to improve the functional quality (SCHMANDKE, 1976; HOFSTEIN & LALASIDIS, 1976) and the nutritive value of food proteins (FUJIMAKI *et al.*, 1977; YAMASHITA *et al.*, 1976).

The plastein reaction requires conditions different from those necessary for the enzymatic hydrolysis of proteins. For instance, the substrate must be a mixture of low- or not too high-molecular peptides, preferably prepared by enzymatic protein hydrolysis (DETERMAN *et al.*, 1963; TSAI *et al.*, 1974; HAJÓS, 1979). The substrate concentration in the plastein reaction should be relatively high, in the range of 20–50 kg $m^{-3}$ (TSAI *et al.*, 1972; HAJÓS, 1979). Also the pH is an influencing factor of plastein formation (YAMASHITA *et al.*, 1971). The pH is strongly dependent on the type of the enzyme used in the plastein reac-

tion. Not all proteolytic enzymes are suitable for the plastein reaction (YAMASHITA *et al.*, 1971). PALLAVICINI and co-workers (1980) used already an immobilised enzyme for plastein synthesis.

The most important among the applications of the plastein reaction is the nutritional improvement of food proteins. By means of the plastein reaction, incorporation of limiting essential amino acids could be made possible, thereby improving the nutritional quality of proteins (FAO/WHO JOINT AD HOC EXPERT COMMITTEE, 1973).

From soy protein hydrolysate ARAI and co-workers (1974) MONTI and JOST (1979) synthesized methionine-enriched plasteins. In our laboratory, the partial hydrolysate of casein was enriched with methionine during the plastein reaction (HAJÓS, 1981).

## 1. Materials and methods

### *1.1. Protein hydrolysate*

Casein was first hydrolysed with papain. This hydrolysis was carried out under the following conditions: casein concentration in the reaction medium: 10 kg $m^{-3}$, enzyme: casein ratio 1 : 100, pH of the reaction system: 1.6, temperature: 37 °C, reaction time: 24 h. The reaction mixture was stirred during the reaction. Then the hydrolysate suspension was adjusted to pH 6.0 with NaOH and a second hydrolysis was carried out with papain under the reaction conditions mentioned above.

### *1.2. Substrate*

The casein hydrolysate (see 1.1.) was centrifuged at 10 000 *g* for 20 min. The soluble fraction was then dialysed through a cellophane membrane to obtain a soluble, non-dialysable fraction as a substrate for plastein synthesis.

### *1.3. Amino acid ester*

L-methionine methyl ester hydrochloride obtained from RIEDEL-DE HAEN AG was used.

### *1.4. Enzymes*

Commercially available pepsin (MERCK), papain (SIGMA), Pronase (SERVA, CALBIOCHEM) and α-chymotrypsin (SIGMA) were used.

### *1.5. Activator*

A reagent grade preparation of L-cystein (REANAL) was used as the activator for papain.

*1.6. Plastein reaction*

The following conditions were applied: substrate concentration: 300 kg $m^{-3}$, enzyme: substrate ratio 1 : 100; incubation temperature: 37 °C, pH: 6.0, incubation time: 48 h. The reaction was carried out without shaking or mixing. A methionine methyl ester : substrate ratio of 5 : 100 was used for the amino acid incorporation process.

*1.7. Determination of amino acid composition*

An aliquot of the samples was hydrolysed with $6 \times 10^3$ mol $m^{-3}$ HCl in an evacuated tube at 105 °C for 24 h. For the more accurate determination of the methionine content, one portion of the hydrolysate was oxidized by performic acid and tested as methionine sulphon. The hydrolysate was analysed for amino acids in a *Beckman* amino acid analyser.

*1.8. Gas chromatography*

The supernatants of the plasteins were extracted by steam distillation and the methanol content was determined in a *Jeol* JGC 1100 gas chromatograph. Stationary phase: *Chromosorb* 103, 100–120 mesh; carrier gas: nitrogen; flow rate: 35 $cm^3$ $min^{-1}$; column length: 2.7 m, diameter 2 mm; detector: FID. Detector and injector temperature: 270 °C.

## 2. Results

*2.1. Incorporation of the* L-*methionine methyl ester into the protein chain during the plastein reaction*

Plastein reaction was carried out with the appropriate substrate (see 1.2.) with and without methionine methyl ester addition under the same conditions. The products were centrifuged (20 000 *g* for 1 h) and the precipitate was dialysed (against distilled water for 24 h). The amino acid content was determined from the sample hydrolysate. A qualitative determination of amino acid composition was done on *Fixion* ion exchange thin layer plates (DÉVÉNYI *et al.*, 1971) (Fig. 1).

On the two edges of the plate, the control amino acids and L-methionine were developed. The three patterns of protein hydrolysates show amino acid compositions of the highest molecular mass fractions of

(1) untreated casein,

(2) plastein without amino acid incorporation, and

(3) plastein with methionine enrichment.

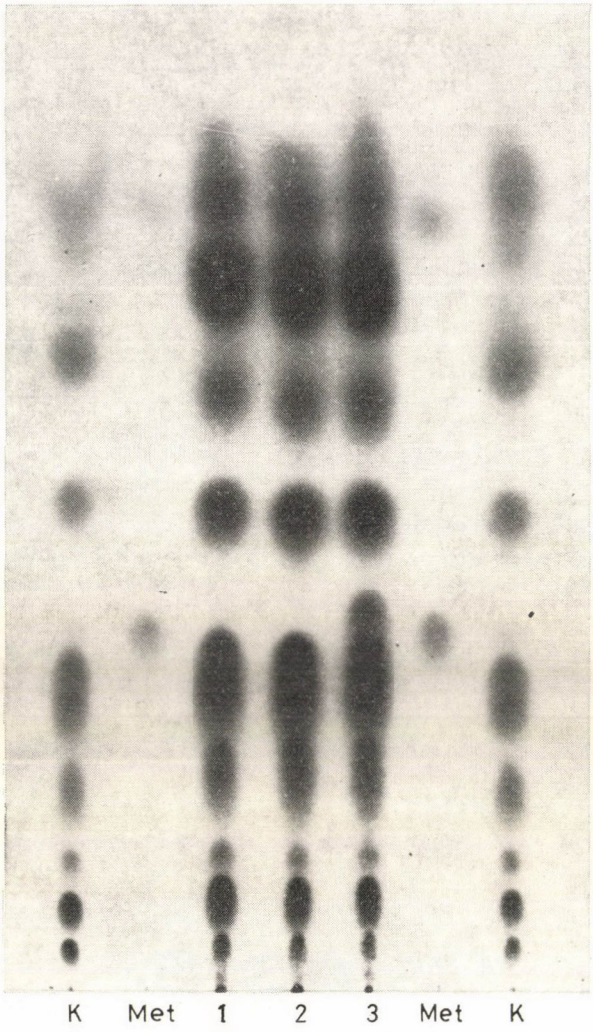


Fig. 1. The amino acid composition of plasteins on *Fixion* plate
K: amino acid mixture (control) Met: methionine

1: casein hydrolysate; 2: hydrolysate of plastein without methionine incorporation; 3: hydrolysate of plastein with methionine incorporation

### *2.2. Determination of the methanol content formed during methionine incorporation in the plastein reaction*

It was to be decided whether the amount of methanol formed by the chemical reaction of methionine incorporation differs significantly from the amount of methanol formed by any side reactions. Therefore a set of reactions as shown in Table 1 was investigated.

Table 1

*The reaction conditions and the methanol content of the set of investigated samples*

| Sample No. | Substrate | Methionine methyl ester | Enzyme | Methanol (%) |
|---|---|---|---|---|
| *1* | + | − | − | 0.03 |
| *2* | − | + | − | 0.02 |
| *3* | − | − | − | 0.01 |
| *4* | + | + | − | 0.18 |
| *5* | − | + | Pronase (CALBIOCHEM) | 0.13 |
| *6* | − | + | papain (SIGMA) | 0.14 |
| *7* | + | + | papain (SIGMA) | 0.38 |
| *8* | + | + | Pronase (CALBIOCHEM) | 0.42 |
| *9* | + | + | α-chymotrypsin (SIGMA) | 0.32 |
| *10* | + | + | Pronase (SERVA) | 0.45 |

By these reactions, the detectable methanol content of *1* the substrate only, *2* methionine methyl ester and *3* the water was investigated. In Experiment *4*, the methanol content of the reaction incubated without addition of any additional enzyme was observed. In reactions *5* and *6*, the interaction of the amino acid ester and the two different enzymes was observed from the point of view of methanol formation.

Methanol content of supernatants of unambiguous plastein reactions were determined with samples *7*, *8*, *9* and *10*.

### *2.3. The methionine content of plastein*

Methionine contents of the samples determined with the amino acid analyser are summarised in Table 2.

Table 2

*Methionine content of the investigated samples*

| Samples | Methionine content (%)[a] |
|---|---|
| *1* control | 2.22 |
| *4* substrate + methionine methyl ester | 7.17 |
| *7* substrate + methionine methyl ester + papain | 12.75 |
| *8* substrate + methionine methyl ester + Pronase (CALBIOCHEM) | 12.90 |
| *9* substrate + methionine methyl ester + α-chymotrypsin | 8.76 |
| *10* substrate + methionine methyl ester + Pronase (SERVA) | 11.46 |

[a] The methionine content of the samples is given as per cent of the total amino acid content

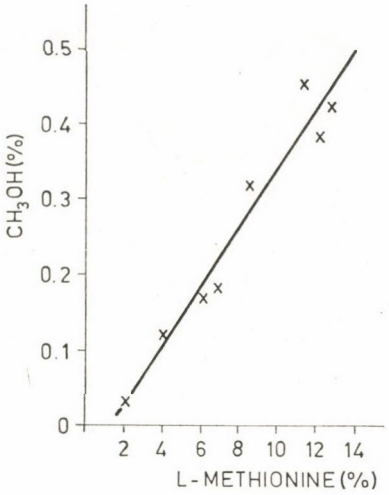


Fig. 2. Investigation of the relationship between methionine content and methanol formation.
$y = 0.11x - 0.06$; $r = 0.946$; $r^2 = 0.895$

*2.4. Investigation of relationship between methionine content and methanol formation*

The data obtained for the methanol content of the samples were plotted against their methionine content. The results were evaluated by linear regression calculation, see Fig. 2.

## 3. Conclusions

*3.1. Improvement of the methionine level of protein*

We have found that methionine methyl ester can be incorporated into the peptide chain by enzymatic resynthesis. The precipitate obtained after the plastein reaction was dialysed. We have shown by control experiments that the higher methionine content can be attributed to methionine incorporation during the enzymatic reaction.

Our results indicate that enzymatic resynthesis constitutes a suitable method for increasing the nutritive value of a given material of high protein content by the incorporation of certain limiting essential amino acids.

*3.2. Investigation of the methanol content formed during methionine incorporation in the enzymatic resynthesis*

The condensation reaction of methionine methyl ester with the peptide chain involves the formation of methanol. We have investigated whether the amount of this methanol is essentially different from the amount of methanol formed in other processes.

In Experiment *1* (see Table 1), casein hydrolysate alone was incubated without the addition of any other reagent. The observed amount of methanol remained below the limits of error.

In Experiment *2*, we wanted to determine the amount of the methanol formed by hydrolysis of methionine methyl ester in aqueous medium at the pH applied in the plastein reaction. This value was found to remain within the allowed limits of error. The methanol contamination of distilled water was also found to be very low, see Experiment *3*.

In Experiment *4*, no additional enzyme was added to the reaction mixture containing methionine methyl ester and the substrate. The observed amount of methanol, however, increased by one order of magnitude. In our opinion, this phenomenon can be explained by the fact that part of the enzyme applied for the hydrolysis of casein and carried over to the substrate, was still active after freezing and freeze drying. Because of this enzyme activity of the sample, in the mixture, a plastein reaction took place to a small extent, thus, amino acid was incorporated, causing a given amount of increase in methanol formation (Table 1).

The possibility of methanol formation due to interaction between amino acid ester and the appropriate enzyme has also been taken into consideration. For this purpose, in Experiments *5* and *6*, methanol was detected from reaction mixtures with methionine methyl ester and Pronase and papain, respectively, incubated at appropriate pH and in the given concentration. The obtained values (see Table 1) were in agreement with our assumption that the amino acid esters undergo polymerisation in the presence of the enzyme.

In Experiments *7–10*, the amount of methanol in the supernatants of unambiguous plasteins were determined, where amino acid was incorporated. On the basis of our data, the methanol values from amino acid incorporating plastein reactions are significantly different from the amount of methanol formed in side reactions (Table 1).

### *3.3. Increasing the methionine content of plasteins by methionine incorporation*

Methionine content of plasteins (Table 2) without methionine incorporation – Sample *1* – proved to be identical with that of the untreated casein. More than two-fold increase of this value was found in Experiment *4* where no additional enzyme was applied, but because of a residual enzyme activity in the substrate, amino acid incorporation took place.

Higher methionine values were found with the unambiguous plastein reactions (Samples *7–10*) where the extent of methionine incorporation changed with the type of the enzyme applied. The highest methionine incorporation was achieved with Pronase: here, the methionine content was increased to a four-fold value.

*3.4. The relationship between the methanol content and the incorporated methionine*

The relationship between the amount of methanol formed during the plastein reaction involved amino acid incorporation and methionine content of the product was established by regression analysis (Fig. 2).

According to the $r$ value, the linear relationship between the measured data, is significant at a probability level of 99.9% (SVÁB, 1967). The value of the coefficient of determination ($r^2$) shows that our original hypothesis, *i.e.* that methanol formation is essentially (about in 90%) due to methionine incorporation, seems to be proved.

Our experiments show that a part of methionine methyl ester can be incorporated into the protein by enzymatic resynthesis. On the basis of the results, we assume that the incorporated amino acid forms a covalent bond to the peptide chain.

*

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Address of the authors:

DR. GYÖNGYI HAJÓS
DR. ANNA HALÁSZ

Central Food Research Institute
H-1022 Budapest, Herman Ottó út 15.
Hungary

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# DETERMINING FAT, PROTEIN AND WATER CONTENT OF PASTRY PRODUCTS BY THE NIR TECHNIQUE

K. J. KAFFKA, K. H. NORRIS and M. ROSZA-KISS

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Detailed studies were made on pastry samples prepared without eggs, with 1, 2, ... 8 whole eggs; the white of 1, 2, ... 8 eggs; the yolk of 1, 2, ... 8 eggs per kg pastry, resp., in order to establish how compositional parameters, especially fat and protein content, can be determined with reflectance measurements in the near infrared wavelength region from 1000 to 2638.4 nm, and to estimate the accuracy in predicting the chemical constituents of pastry products using the instrumental method mentioned.

Samples of different fractions of particle size with identical composition were also prepared to determine how this disturbing factor influences measurements.

A "one of a kind" computerized spectrophotometer built at USDA BARC INSTRUMENTATION RESEARCH LABORATORY, Beltsville, was used to study the pastry samples specially prepared at the RESEARCH INSTITUTE OF FLOUR MILLING AND BAKING INDUSTRIES, Budapest. Here these pastry samples were also labelled with the compositional parameters determined by traditional standard methods.

Near infrared reflectance factor (R) spectra were recorded for 90 pastry samples. The spectra were then transformed to log (1/R) versus wavelength and to the second derivative of log (1/R) versus wavelength for correlation with compositional data. Multiple linear stepwise regression techniques were used to determine the optimum wavelengths and other parameters for predicting each of the chemical constituents.

The NIR technique applied to pastry–averaging the measurements for three replicates–provides one-term prediction equation giving correlation coefficients of 0.9973 for fat, 0.9863 for protein, and 0.9622 for water. In a measurement time of less than 6 minutes for the three replicates of a pastry sample, it was possible to determine fat content of pastry samples within a standard error of calibration of $\pm$ 0.112 mass %, protein content within $\pm$ 0.239 mass %, and water content within 0.243 mass %.

The possible application and limitation of the NIR technique as applied to pastry were discussed. We concluded that the NIR technique has the potential for use in rapid evaluation of pastry quality.

The ever increasing demands for constant and improved quality of food products and especially of pastry products requires the development of instruments for the rapid determination of quality parameters.

Fat, protein, and water content are the most significant quality parameters of pastry from which the egg content can be determined in an easy way. The analytical methods for determining the above parameters are described in respective HUNGARIAN STANDARDS (1972, 1979, 1980).

Accurate analyses for fat, protein, and moisture in pastry products by traditional methodology is a slow process requiring special chemicals and sever-

al hours before test results are available. The time required for these chemical analyses is too long for automatic control of production. On the other hand, the time and working power demands also hinder the application of these methods for the control of quality of pastry products in the shops.

References on papers dealing with the ways to determine the composition of the raw materials of pastry products using the near infrared reflection (NIR) technique, the instruments used, the comparison of the results achieved with the NIR technique and those obtained with traditional analytical methods, and disturbing factors influencing the results have been reported by KAFFKA and KULCSÁR (1982).

In this study, the authors drew the conclusion that in the case of pastry products, the egg content can be predicted with a quick, accurate, and non-destructive instrumental method using near infrared reflection data measured at some characteristic wavelengths. It was, however, established that further experiments were to be performed in order to determine the fat and protein content separately in such pastry samples (specially prepared) where fat and protein content vary independently.

This study aims at finding the possibility for applying the NIR technique for rapid determination of fat, protein, and even egg content in pastry products; and at the same time to predict accuracy for the different components, to choose the most suitable form of the regression equations, determine the parameters (characteristic wavelengths, coefficients, and constants), and last, but not least, to examine the achievable accuracy increase by studies on the transformed reflection spectra.

The results of these studies have lead to the possibility of developing a single-purpose instrument.

The timeliness of our research work was stressed by the fact that in order to modify and to modernize the respective items of HUNGARIAN STANDARD (1972) a multiple examination is in process to compare the methods used so far in determining the egg content in different pastry products as macaroni, spaghetti, vermicelli, *etc.*

## 1. Materials and methods

Pastry products (also called pastas) are made of flour and water with or without eggs by mixing, kneading, rolling, or forming, respectively, then drying. The relevant Hungarian standards for pastry products were listed in the above-mentioned preliminary study.

Since the NIR technique is strictly correlative, the calibration samples are critically important, and the calibration samples should include all the variability in composition (range of constituent values), particle size, sample treatment, *etc.*, that might be encountered in any sample in the practice to be

measured. Taking these all into consideration, the pastry samples for calibration were carefully prepared at the RESEARCH INSTITUTE OF FLOUR MILLING AND BAKING INDUSTRIES, Budapest.

The pastry samples used for our examination were made of TL 50 wheat flour corresponding to HUNGARIAN STANDARD (1968). This means ash content is less than 0.5 mass %, wet gluten minimum 28 mass %, moisture content less than 15.2 mass %, and particle size between 200–400 $\mu$m (maximum 15% below 200 $\mu$m). The fresh whole eggs used corresponded to the requirements in HUNGARIAN STANDARD (1978).

Carefully analyzed ingredients of pastry: flour, egg white (albumen), and egg yolk, as well as water, were mixed in predetermined quantities so that the composition of the pastry samples could be calculated. The composition of the different samples were reanalyzed to make sure that calculated data corresponded to analytical data.

The dry matter content (moisture content) was determined on the basis of the corresponding HUNGARIAN STANDARD (1972). The protein content was determined using an automatic *Kjel-Foss* 162–10 instrument. Fat content determination was performed with a modified version of the *Lidner* method. This method is based on weighing the mass of the residual obtained after digestion with phosphoric acid – alcohol, extraction with petroleum ether, and evaporation of the solvent.

Before measuring the optical properties, the pastry products were ground in a continuously working hammer mill (FALLING NUMBER INC.), and the fraction of grain size below 200 $\mu$m was used for samples numbered from No. 1 to No. 25, while the other fractions with very different sizes were used for samples from No. 26 to No. 30, in order to see the influence of particle size and to be able to compensate it.

Samples from No. 26 to No. 30 were the same composition as sample No. 25, but the particle size was above 250 $\mu$m for No. 26, below 250 $\mu$m for No. 27, below 200 $\mu$m for No. 28, below 160 $\mu$m for No. 29, and below 125 $\mu$m for sample No. 30.

We placed 10 $cm^3$ of powdered pastry sample into the sample holder (cell) of 38 mm diameter with a 1.2 mm thick special quartz window (type G. E. 124) on one side and pressed with a pressure of about 1 mPA from the other side. Illumination was done with a beam (the cross-section was not quite circular), the diameter of which varied between 15 to 20 mm at an incident angle of 0° through the quartz window (the illumination was perpendicular to the surface of the sample). Four lead sulfide detectors (sensors) were equally spaced around the incident beam to measure the radiation diffusely reflected by the sample at 45°.

Each of the 30 different pastry samples prepared at the RESEARCH INSTITUTE OF FLOUR MILLING AND BAKING INDUSTRIES, Budapest, were packed

into, three cells, thus we got a total of 90 samples to analyze and to use for calibration. Having three replicates from the 30 original samples, we had the opportunity to reduce the errors by averaging the measurements.

The near infrared reflection properties of pastry samples were measured with a "one of a kind" computerized spectrophotometer built at USDA BARC INSTRUMENTATION RESEARCH LABORATORY, Beltsville. This instrument is built around a *Cary Model* 14 prism-grating monochromator with optics optimized for the near infrared. The monochromator is coupled to a digital computer for collecting and analysis of data. The instrument is operated in a single-beam mode with a reference spectrum stored in the computer.

The monochromator was operated with slits at 2 mm giving an effective bandpass of 7 nm. The region scanned was 1 000 to 2 638.4 nm. The signal from the lead sulfide detectors were amplified, digitized, and fed to the digital computer. Reflection data were collected every 0.2 nm with 256 digital conversions per point. Thus, we got 8 192 reflection points for the whole wavelength region. The scanning speed was 10 nm per second, so the time period between two data collections was 20 ms; with one half of the time for 256 readings during the "light period", the other half to measure the "dark period" signal from the AC coupled amplifier in order to restore the absolute amplitude. To take a complete reflection spectrum of a sample required about 165 seconds.

Ceramic material was used as a reference standard because its reflectance is the same for all wavelengths from 1 000 nm to 2 700 nm. The reflected signal from the ceramic standard was stored in the computer and used to divide the reflected signal from the pastry samples. This gives a resultant curve of true diffuse directional-conical reflectance relative to the ceramic standard. (The term directional-conical reflectance is meant as a ratio of reflected flux collected through a conical solid angle to essentially collimated incident flux.) The computer corrects for the reflectance of the ceramic standard to give the real value of the diffuse directional-conical reflectance factor (R); the ratio of the flux actually reflected by a sample surface to that which would be reflected into the same reflected-beam geometry by an ideal (lossless), perfectly diffuse (lambertian) standard surface irradiated in exactly the same way as the sample. The diffuse directional-conical reflectance factor data were recorded at each measured reflection point for each of the pastry samples, and the spectral data were stored on magnetic tapes for further processing. A new reference signal from the ceramic standard was stored in the computer about once each hour to minimize errors from long-term drift.

For data processing, the 8 192-point spectral curves were smoothed by a running average of 21 points at each point and shrunk to 1 024-point curves by choosing every eighth point and averaging it with adjacent points on either side. Compressed spectral curves were transformed to log (1/R) curves and recorded along with the compositional data for each sample. The transforma-

tion of the R spectral curves to log (1/R) proved useful because the log (1/R) function gives a linear correlation with the concentration of a given measured component. Plotting log (1/R) as a function of wavelength gives a spectral curve that is comparable to an absorption curve having peak readings at wavelengths that correspond to absorption bands in the sample. Preliminary studies indicated that performance could be improved by using the second derivative of the log (1/R) spectral curve rather than log (1/R). Therefore, all data were analyzed by use of the second derivative transformation of each wavelength point. The second derivative calculation was incorporated into the computer program for the linear regression analysis. A stepwise multiple-linear-regression program was used also to analyze the data and determine the optimum wavelengths and other important parameters for predicting fat, protein, and water content in pastry samples.

Because changes in reflectance are small, even from relatively large changes in composition, reflectance data must be recorded with high precision. For this reason, the resolution we used at the measurements was 0.0000305 log (1/R) unit [1 log (1/R) corresponds to 32 768 steps].

The definition of the standard error of calibration used in the discussion is:

$$\sqrt{\frac{\Sigma (Q_{si} - Q_{ci})^2}{n-1-p}}$$

where

$Q_s$ is the respective quality parameter (dependent variable) determined by traditional standard methods,

$Q_c$ is the same quality parameter computed from the regression equation,

$n$ is the number of samples,

$p$ is the number of independent variables.

The standard error of calibration is derived by comparing the laboratory values of one set of samples to the instrument values of that same set when creating the calibration equation. The standard error of prediction is obtained by comparing the laboratory values of a second set of samples to the instrument percent reading of that second set and verifying the existing calibration.

All measurements described were carried out in an air-conditioned laboratory at 296 K (+23 °C).

## 2. Results

The quantities of ingredients, such as wheat flour, white of egg, and yolk of egg used in preparing the 30 pastry samples, are summarized in Table 1.

The compositional data – namely fat, protein, and water content – of the 30 pastry samples are summarized in Table 2.

Table 1

*The data of ingredients of the 30 pastry samples*

| Sample No. | Wheat flour (g) | White of egg (g) | Yolk of egg (g) |
|---|---|---|---|
| 1 | 515 | — | — |
| 2 | 512 | 17 | — |
| 3 | 510 | — | 8 |
| 4 | 509 | 34 | — |
| 5 | 505 | — | 16 |
| 6 | 506 | 51 | — |
| 7 | 501 | — | 24 |
| 8 | 503 | 68 | — |
| 9 | 496 | — | 32 |
| 10 | 500 | 85 | — |
| 11 | 492 | — | 40 |
| 12 | 497 | 102 | — |
| 13 | 487 | — | 48 |
| 14 | 494 | 119 | — |
| 15 | 482 | — | 56 |
| 16 | 492 | 136 | — |
| 17 | 476 | — | 64 |
| 18 | 507 | 17 | 8 |
| 19 | 500 | 34 | 16 |
| 20 | 492 | 51 | 24 |
| 21 | 484 | 68 | 32 |
| 22 | 477 | 85 | 40 |
| 23 | 469 | 102 | 48 |
| 24 | 462 | 119 | 56 |
| 25 | 454 | 136 | 64 |

We selected the log (1/R) spectra of three pastry samples made with 0, 4, and 8 whole eggs per kg pastry which are shown in Fig. 1.

We selected also the log (1/R) spectra of three samples made with the yolk of 0, 4, and 8 eggs per kg pastry which are shown in Fig. 2.

From the spectra in Fig. 1 and Fig. 2, it can be seen that the higher the egg or yolk content, the lower the log (1/R) values; that is, the reflected flux increases with an increase in egg or yolk content. The contribution of the white of egg in shifting the log (1/R) spectral curves downward is relatively small. It can also be noticed that decrease in log (1/R) values are not proportional with the increase of egg or yolk content; the differences between the log (1/R) values are decreasing with adding stepwise more and more egg or yolk.

Table 2

*The compositional data of the 30 pastry samples*

| Sample No. | Protein cont. (mass %) | Fat cont. (mass %) | Water cont. (mass %) |
|---|---|---|---|
| 1 | 12.46 | 1.030 | 10.21 |
| 2 | 12.83 | 1.034 | 9.745 |
| 3 | 12.56 | 1.527 | 9.255 |
| 4 | 13.19 | 1.038 | 10.15 |
| 5 | 12.74 | 2.024 | 9.190 |
| 6 | 13.55 | 1.043 | 12.23 |
| 7 | 12.90 | 2.524 | 9.640 |
| 8 | 13.91 | 1.047 | 10.36 |
| 9 | 13.03 | 3.021 | 11.23 |
| 10 | 14.28 | 1.051 | 10.92 |
| 11 | 13.19 | 3.520 | 11.84 |
| 12 | 14.64 | 1.055 | 11.74 |
| 13 | 13.33 | 4.017 | 11.16 |
| 14 | 15.00 | 1.059 | 12.05 |
| 15 | 13.47 | 4.514 | 11.87 |
| 16 | 15.39 | 1.066 | 11.98 |
| 17 | 13.58 | 5.009 | 11.30 |
| 18 | 12.96 | 1.531 | 12.76 |
| 19 | 13.49 | 2.049 | 11.44 |
| 20 | 13.98 | 2.509 | 11.89 |
| 21 | 14.48 | 3.038 | 11.50 |
| 22 | 15.01 | 3.541 | 11.72 |
| 23 | 15.51 | 4.042 | 11.68 |
| 24 | 16.03 | 4.546 | 10.98 |
| 25 | 16.53 | 5.047 | 10.96 |
| 26 | 16.53 | 5.047 | 10.96 |
| 27 | 16.53 | 5.047 | 10.96 |
| 28 | 16.53 | 5.047 | 10.96 |
| 29 | 16.53 | 5.047 | 10.96 |
| 30 | 16.53 | 5.047 | 10.96 |

The spectra shown in Fig. 1 and Fig. 2 were recorded for samples having the same granularity (particle size distributions); while Fig. 3 shows spectra for samples with the same composition but with different granularity.

Comparison of the spectra of Fig. 1 and Fig. 2 with the spectra of Fig. 3 shows that change in the particle size might shift the spectra vertically in the

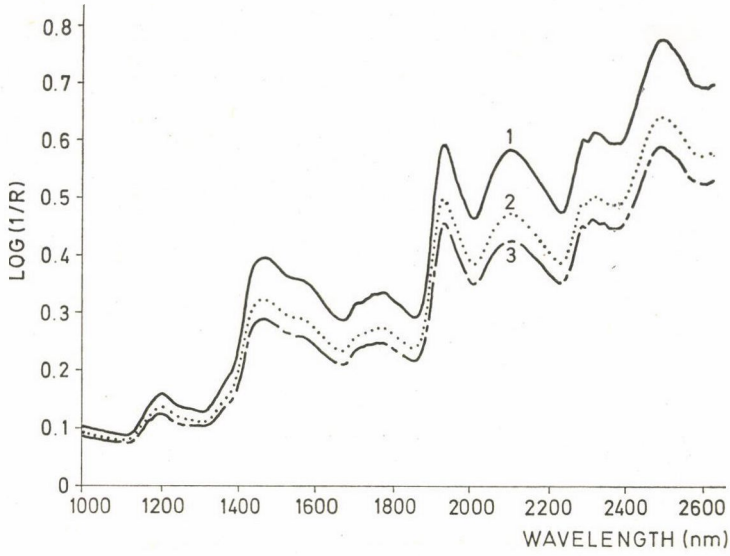


Fig. 1. Typical log (1/R) spectra of pastry samples. The measuring geometry: 0°/45°. Diameter of the illuminating beam: 20/15 mm. Spectral bandpass: 7 nm. Spectra were taken in 0.2 nm steps, compressed, then recorded in 1.6 nm steps. Vertical resolution: 0.00003 log (1/R) unit.
1: Pastry sample No. 1 made without eggs; 2: Pastry sample No. 21 made with four eggs per kg pastry; 3: Pastry sample No. 25 made with eight eggs per kg pastry

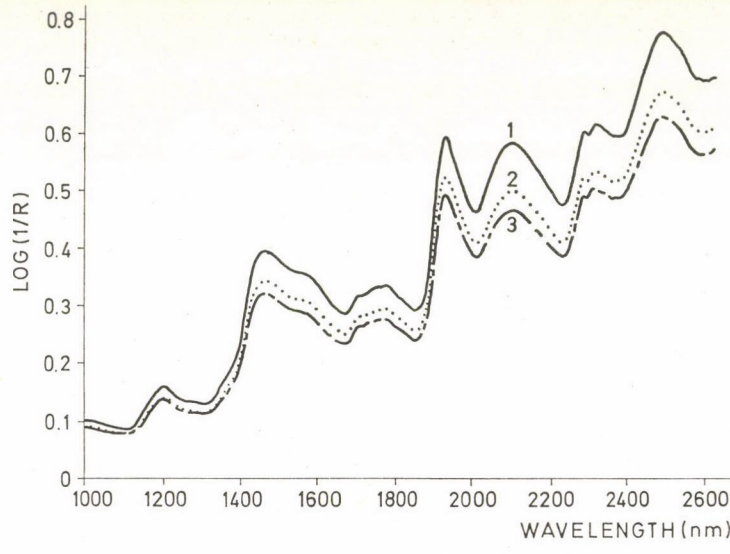


Fig. 2. Typical log (1/R) spectra of pastry samples. The measuring geometry: 0°/45°. Diameter of the illuminating beam: 20/15 mm. Spectral bandpass: 7 nm. Spectra were taken in 0.2 nm steps, compressed, then recorded in 1.6 nm steps. Vertical resolution: 0.00003 log (1/R) unit.
1: Pastry sample No. 1 made without eggs; 2: Pastry sample No. 9 made with the yolk of 4 eggs per kg pastry; 3: Pastry sample No. 17 made with the yolk of 8 eggs per kg pastry

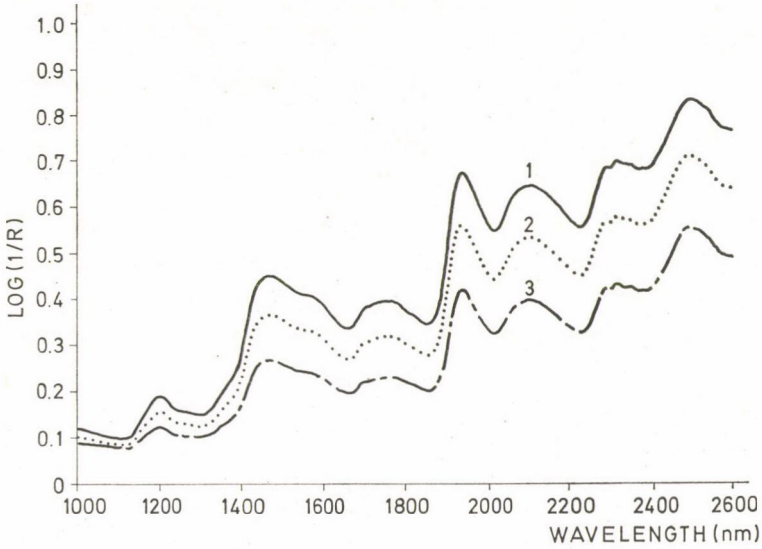


Fig. 3. The log (1/R) spectra of pastry samples with the same composition made with eight eggs per kg pastry but different particle size. The measuring geometry: 0°/45°. Diameter of the illuminating beam: 20/15 mm. Spectral bandpass: 7 nm. Spectra were taken in 0.2 nm steps, compressed, then recorded in 1.6 nm steps. Vertical resolution: 0.00003 log (1/R) unit.
1: Pastry sample No. 26 with particle size above 250 $\mu$m; 2: Pastry sample No. 27 with particle size below 250 $\mu$m; 3: Pastry sample with particle size below 125 $\mu$m

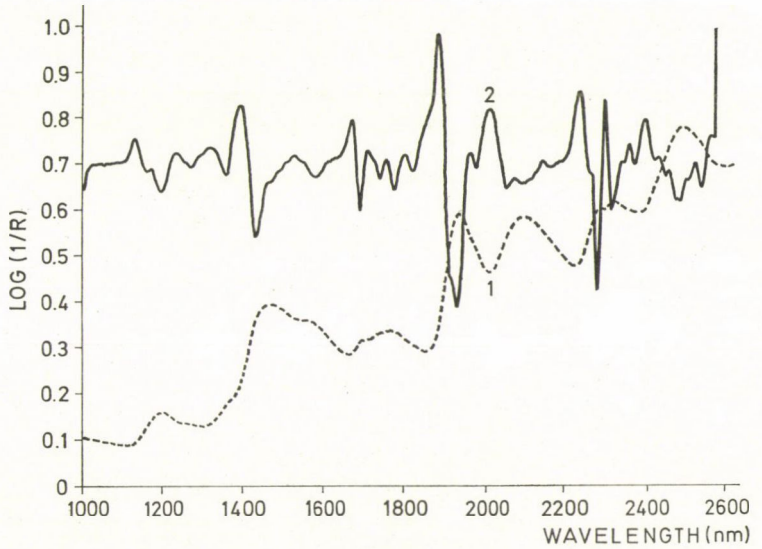


Fig. 4. The log (1/R) spectrum and the second derivative curve of pastry sample No. 1 made without eggs.
1: Log (1/R) spectrum; 2: Second derivative of the log (1/R) spectrum

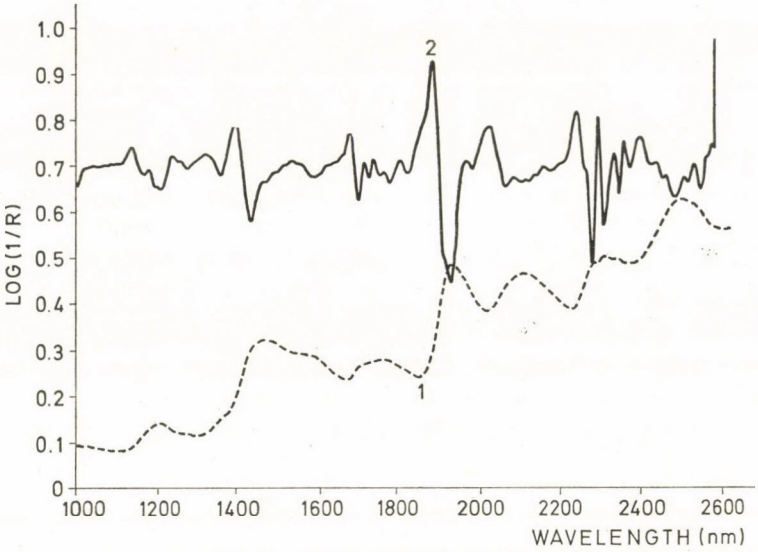


**Fig. 5.** The log (1/R) spectrum and the second derivative curve of pastry sample **No. 17** made with the yolk of 8 eggs per kg pastry

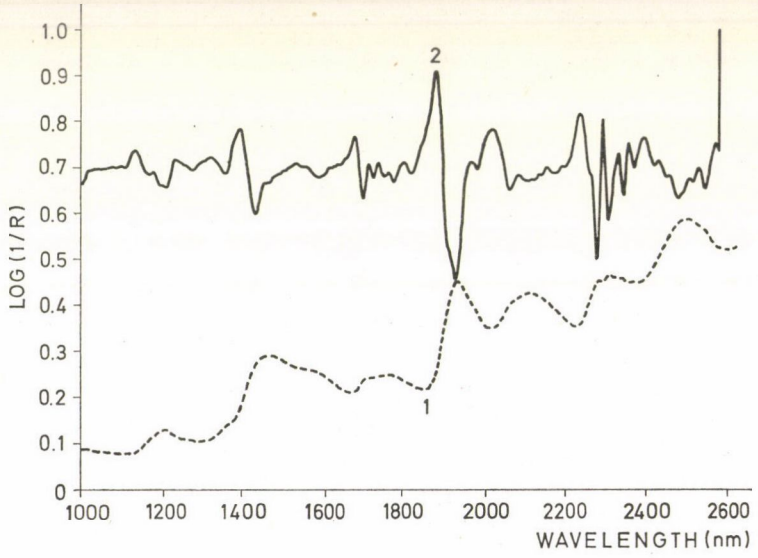


**Fig. 6.** The log (1/R) spectrum and the second derivative curve of pastry sample **No. 25** made with eight whole eggs

same considerable manner than the composition does. The increase in the particle size increases the log (1/R) values. The log (1/R) spectra and their second derivative curves for pastry samples made without eggs, with the yolk of 8 eggs, and with 8 whole eggs per kg pastry, resp., are seen in Fig. 4, Fig. 5 and Fig. 6.

The figures illustrate that the second derivative of the spectra do show a much greater difference than log (1/R) between samples sharpening the details in the curves.

The relationship between log (1/R) values and fat, protein, and water content of the 90 pastry samples was determined by use of an iterative procedure repeated for up to four steps with the multiple regression analyses. The computer program determined the characteristic wavelengths and the coefficients from $K_0$ to $K_4$ that gave the best fit to the following equation for each measured component:

$$Q_{1i} = K_{0i} + K_{1i} V_{\lambda 1i} + K_{2i} V_{\lambda 2i} + K_{3i} V_{\lambda 3i} + K_{4i} V_{\lambda 4i}$$

where $Q_{1i}$ stands for the composition parameters (fat, protein, and water content); $K_{0i}, K_{1i}, \ldots K_{4i}$ are coefficients and constants, respectively; $\lambda_{1i}, \ldots \lambda_{4i}$ are characteristic wavelengths; $V_{\lambda 1i}, \ldots V_{\lambda 4i}$ are the log (1/R) values belonging to these characteristic wavelengths.

The results are summarized for fat, protein, and water content in Table 3, containing the characteristic wavelengths, the coefficients and constants of regression equation, as well as standard error of calibration.

We tried to find the relationship between composition parameters and log (1/R) values at other absorption bands of the above-mentioned components using the same equation form. The results were essentially the same as in Table 3; we summarized them in Table 4.

Following this, we endeavored to increase accuracy by transforming the data to the second derivative of the log (1/R) spectra. The relationship between transformed spectral data and composition parameters was studied in the following form of equations:

$$Q_{2i} = K_{5i} + K_{6i} \frac{V''_{\lambda 5i}}{V''_{\lambda 6i}}$$

where $Q_{2i}$ stands for the composition parameters; $K_{5i}$, $K_{6i}$ are coefficients; $V''_{\lambda 5i}$ and $V''_{\lambda 6i}$ are the values of the second derivatives of the log (1/R) spectra at $\lambda_{5i}$ and $\lambda_{6i}$ characteristic wavelengths.

Characteristic wavelengths, constants, and coefficients of the regression equations, as well as standard error of calibration — using this single term equation — are summarized in Table 5.

In this table, the gaps for numerator and denominator, wavelengths, as well as the segments, are listed. The gaps are the distances among the three

Table 3

*Summary of multiple linear regression analyses relating data from chemical analyses and log (1/R) values measured at four characteristic wavelengths for 90 pastry samples, searching 1 000 to 2 500 nm*

Equation form: $Q_{1i} = K_{0i} + K_{1i}V_{\lambda 1i} + K_{2i}V_{\lambda 2i} + K_{3i}V_{\lambda 3i} + K_{4i}V_{\lambda 4i}$

| | For fat content | For protein content | For water content |
|---|---|---|---|
| Characteristic wavelength $\lambda_1$ (nm) | 1 779.2 | 1 896.0 | 1 406.4 |
| Characteristic wavelength $\lambda_2$ (nm) | 1 764.8 | 1 662.4 | 1 390.4 |
| Characteristic wavelength $\lambda_3$ (nm) | 1 409.6 | 1 680.0 | 1 398.4 |
| Characteristic wavelength $\lambda_4$ (nm) | 1 744.0 | 1 697.6 | 1 337.6 |
| Constant $K_0$ | 7.8750 | 24.328 | 12 .063 |
| Coefficient $K_1$ | −1 455.0 | 24.089 | −1 799.7 |
| Coefficient $K_2$ | 1 858.8 | −3 162.7 | −3 001.4 |
| Coefficient $K_3$ | 59.944 | 4 276.4 | 4 643.2 |
| Coefficient $K_4$ | −459.49 | −1 132.0 | 156.81 |
| Standard error of calibration (mass %) | 0.186 | 0.313 | 0.280 |
| Multiple correlation coefficient | 0.9936 | 0.9762 | 0.9490 |

Table 4

*Summary of multiple linear regression analyses relating data from chemical analyses and log (1/R) values measured at four characteristic wavelengths for 90 pastry samples, searching in selected wavelength regions*

Equation form: $Q_{1i} = K_{0i} + K_{1i}V_{\lambda 1i} + K_{2i}V_{\lambda 2i} + K_{3i}V_{\lambda 3i} + K_{4i}V_{\lambda 4i}$

| | For fat content | For protein content | For water content |
|---|---|---|---|
| Characteristic wavelength $\lambda_1$ (nm) | 2 275.2 | 2 120.0 | 1 936.0 |
| Characteristic wavelength $\lambda_2$ (nm) | 2 300.8 | 2 049.6 | 2 286.4 |
| Characteristic wavelength $\lambda_3$ (nm) | 2 222.4 | 2 043.2 | 2 321.6 |
| Characteristic wavelength $\lambda_4$ (nm) | 1 745.6 | 2 070.4 | 2 168.0 |
| Constant $K_0$ | 14.173 | 16.373 | 16.404 |
| Coefficient $K_1$ | −832.10 | −563.57 | 86.470 |
| Coefficient $K_2$ | 665.39 | 3 111.8 | 492.92 |
| Coefficient $K_3$ | 149.73 | −2 283.3 | −539.03 |
| Coefficient $K_4$ | 28.179 | −267.26 | −36.599 |
| Standard error of calibration (mass %) | 0.191 | 0.316 | 0.295 |
| Multiple correlation coefficient | 0.9933 | 0.9757 | 0.9434 |

wavelengths used for producing the second derivative, while segment is the wavelength region in which the measured spectral data are averaged.

The protein, water, and fat have absorption bands in several regions of the near infrared, so we tested selected regions. The results, using other characteristic wavelength sets, are summarized in Table 6.

Table 5

*Summary of linear regression analyses relating data from chemical analyses and values of the second derivative of log (1/R) curves at two characteristic wavelengths for 90 pastry samples, searching 1 000 to 2 500 nm*

Equation form: $Q_{si} = K_{si} + K_{si}(V''\lambda_{si}/V''\lambda_{si})$

| | For fat content | For protein content | For water content |
|---|---|---|---|
| Characteristic wavelength $\lambda_5$ (nm) | 2 310.4 | 1 404.8 | 1 467.2 |
| Characteristic wavelength $\lambda_6$ (nm) | 1 892.8 | 2 054.4 | 1 401.6 |
| Constant $K_5$ | −0.87482 | 25.140 | −0.99096 |
| Coefficient $K_6$ | −6.7141 | 3.5716 | −14.636 |
| Segment (wvl. range for averaging) (nm) | 6.4 | 9.6 | 6.4 |
| Gap for numerator (nm) | 19.2 | 17.6 | 17.6 |
| Gap for denominator (nm) | 11.2 | 12.8 | 11.2 |
| Standard error of calibration (mass %) | 0.122 | 0.268 | 0.329 |
| Correlation coefficient | −0.9972 | 0.9820 | −0.9261 |

Table 6

*Summary of linear regression analyses relating data from chemical analyses and values of the second derivative of log (1/R) curves at two characteristic wavelengths for 90 pastry samples, searching in selected wavelength regions*

Equation form: $Q_{si} = K_{si} + K_{si}(V''\lambda_{si}/V''\lambda_{si})$

| | For fat content | For protein content | For water content |
|---|---|---|---|
| Characteristic wavelength $\lambda_5$ (nm) | 1 721.6 | 2 073.6 | 2 019.2 |
| Characteristic wavelength $\lambda_6$ (nm) | 1 643.2 | 2 054.4 | 1 918.4 |
| Constant $K_5$ | 4.1461 | 22.673 | 35.875 |
| Coefficient $K_6$ | −3.0185 | −4.5504 | 44.554 |
| Segment (wvl. range for averaging) (nm) | 3.2 | 9.6 | 6.4 |
| Gap for numerator (nm) | 17.6 | 19.2 | 12.8 |
| Gap for denominator (nm) | 19.2 | 11.2 | 11.2 |
| Standard error of calibration (mass %) | 0.128 | 0.344 | 0.340 |
| Correlation coefficient | −0.9969 | −0.9701 | 0.9211 |

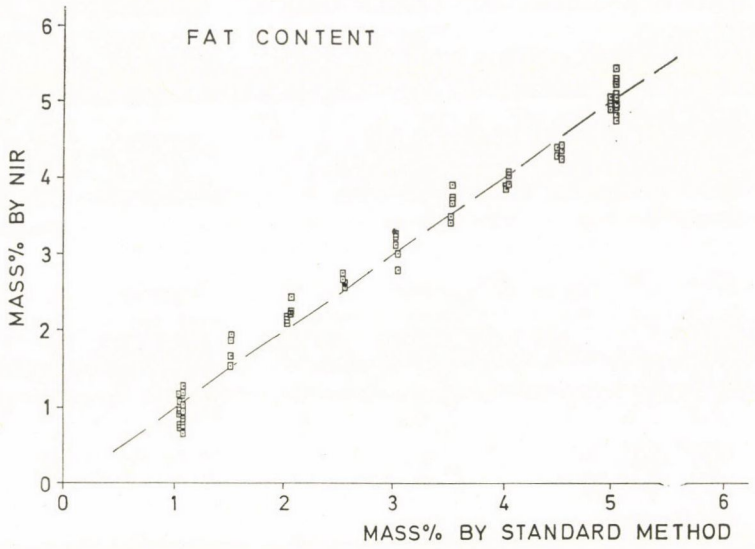


Fig. 7. Relationship between fat content determined by chemical analysis and predicted value from multiple regression log (1/R) data at four wavelengths

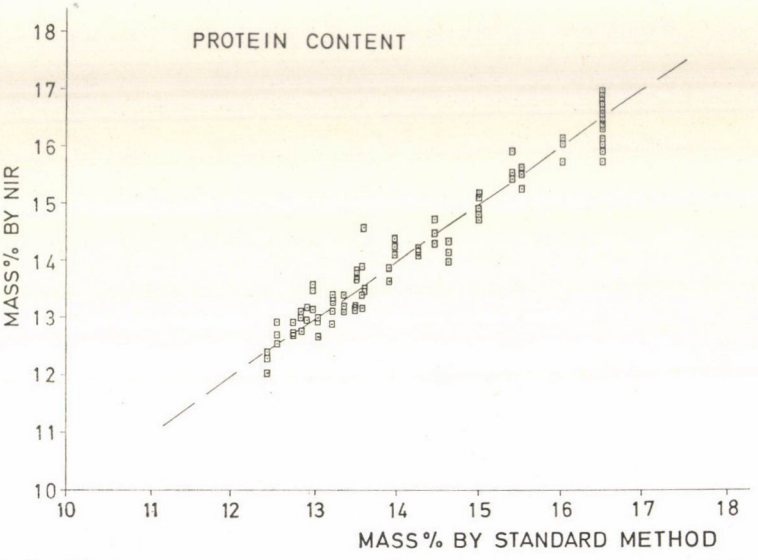


Fig. 8. Relationship between protein content determined by chemical analysis and predicted value from multiple regression log (1/R) data at four wavelengths

The best results for fat, protein, and water determination using log (1/R) values and the second derivative ratio — shown in Table 3 and Table 5 — are graphed in Figures 7 through 12.

Averaging the three replicates for the thirty original samples, a slightly better result can be achieved. The results are summarized in Table 7; only

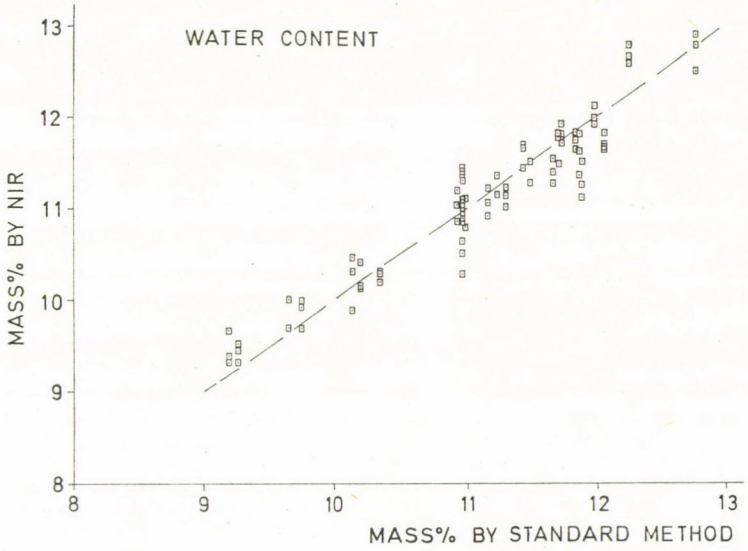


Fig. 9. Relationship between water content determined by chemical analysis and predicted value from multiple regression log (1/R) data at four wavelengths

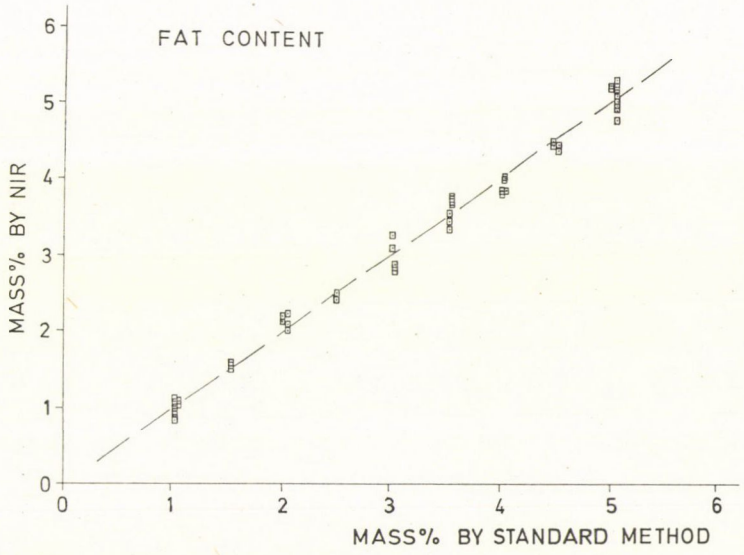


Fig. 10. Relationship between fat content determined by chemical analysis and predicted value from linear regression of the ratio of the second derivatives of the log (1/R) curves at two wavelengths

the standard errors of calibration and the multiple correlation coefficients are listed, all the other conditions are the same as in Table 3 and Table 5.

The reproducibility was determined by comparing the spectra from the three replicates of the original thirty samples. Reproducibility was character-

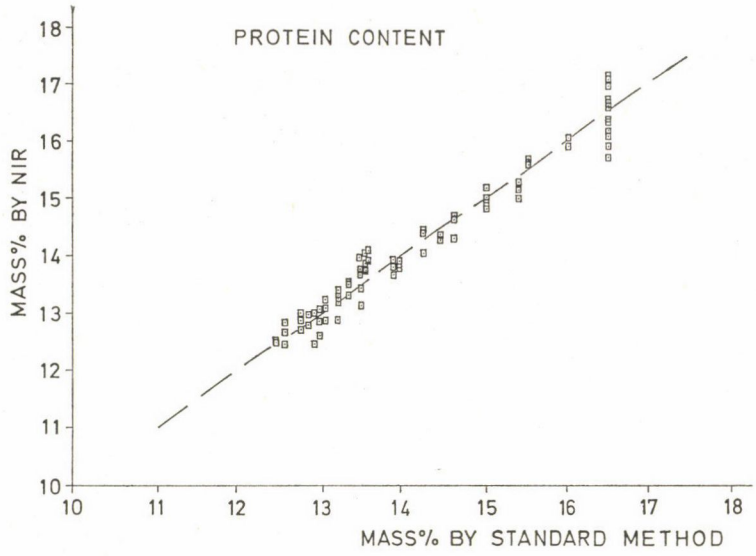


Fig. 11. Relationship between protein content determined by chemical analysis and predicted value from linear regression of the ratio of the second derivatives of the log (1/R) curves at two wavelengths

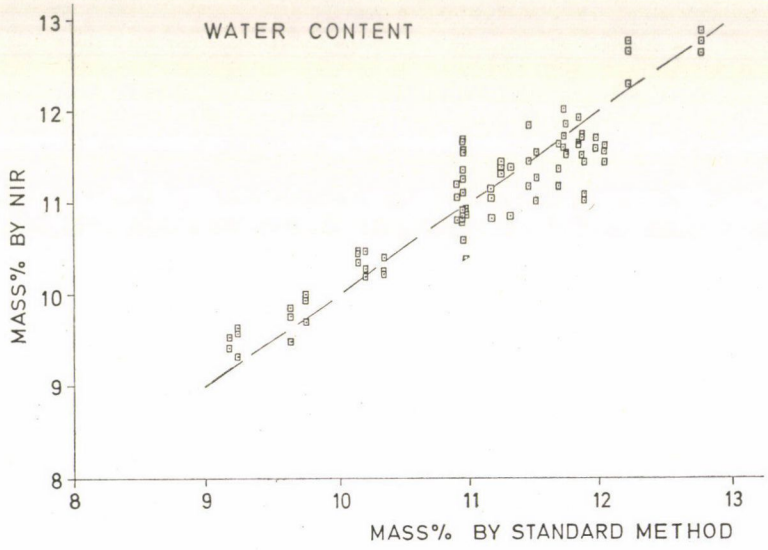


Fig. 12. Relationship between water content determined by chemical analysis and predicted value from linear regression of the ratio of the second derivatives of the log (1/R) curves at two wavelengths

ized by the average of the standard deviations of the predicted data of the values which can be seen for the different components and different equation forms in Table 8.

Table 7

*Summary of multiple linear regression analyses relating data from chemical analyses and log (1/R) values (1) measured at four characteristic wavelengths as well as the second derivatives of log (1/R) curves (2) at two characteristic wavelengths using the average of the three replicates of the 30 original pastry samples*

| | Standard error (mass %) | $R$ |
|---|---|---|
| $Q_{1FAT}$ | 0.184 | 0.9945 |
| $Q_{2FAT}$ | 0.112 | 0.9973 |
| $Q_{1PROTEIN}$ | 0.254 | 0.9863 |
| $Q_{2PROTEIN}$ | 0.239 | 0.9863 |
| $Q_{1WATER}$ | 0.237 | 0.9680 |
| $Q_{2WATER}$ | 0.243 | 0.9622 |

Table 8

*Reproducibility and repeatability for pastry samples expressed as the average of the standard deviations (SD) of the predicted data for fat, protein, and water using multiterm log (1/R) (1) and second derivative (2) forms of equations*

| | Reproducibility average SD (mass %) | Repeatability average SD (mass %) |
|---|---|---|
| $Q_{1FAT}$ | 0.084 | 0.059 |
| $Q_{2FAT}$ | 0.057 | 0.045 |
| $Q_{1PROTEIN}$ | 0.217 | 0.118 |
| $Q_{2PROTEIN}$ | 0.137 | 0.094 |
| $Q_{1WATER}$ | 0.203 | 0.159 |
| $Q_{2WATER}$ | 0.204 | 0.159 |

The repeatability was determined by recording 10 spectral scans on each of three selected samples without changing the position of the samples. The selected samples were those made with 0, 4, and 8 eggs per kg pastry. The repeatability was characterized by the average of the standard deviations of the predicted data of 10 scans for the three samples. The repeatability data for the different components and different equation forms are shown in Table 8.

To determine egg content, the following equation gives the best fit to the chemical analysis:

$$Q_{EGG} = 3.0101 + 12.555 \frac{V''_{\lambda 7}}{V''_{\lambda 8}}$$

where $V''_{\lambda 7}$ and $V''_{\lambda 8}$ are the values of the second derivatives of log (1/R) spectra at 2 331.2 nm and 2 257.6 nm wavelengths.

Using this equation, it was possible to determine egg content of pastry samples within a standard error of 0.086 egg unit.

## 3. Conclusions

As can be seen in Table 2, the fat in our pastry samples has a range of 1.030–5.046 mass %; the change is about 400% in fat content.

The range of protein is from 12.460 to 16.029 mass %; the change is 28% in protein content.

For moisture, the range is from 9.19 to 12.76 mass %; so the change is 38% in water content.

The range of particle size was very broad; samples with particle sizes below 125 $\mu$m up to particle sizes above 250 $\mu$m were studied.

Although the variation in the range of composition and particle size was very broad, good results were obtained for fat and protein content of pastry samples using the NIR technique. Our samples were not prepared as calibration samples for moisture determination, but acceptable results were obtained for moisture. In all cases, no samples were omitted for the calculations, although the results could have been improved by omitting samples with large error.

Our experiments showed satisfactory accuracy for multi-term linear equations containing the log (1/R) values measured at different characteristic wavelengths.

Far better accuracy was obtained with the transformation of log (1/R) spectra; namely by using the second derivatives of the spectra. In this case, we actually used a single term equation where the value of the second derivative of the log (1/R) spectra at the first characteristic wavelengths was divided with the value of the second derivative of the log (1/R) spectra at the second characteristic wavelength.

In a preliminary study, KAFFKA and KULCSÁR (1982) reported achieving good results with 25 pastry samples where by changing only the egg content, the fat and protein contents vary at the same rate. In this report, it was stated that further experiments are to be performed for determining the fat and protein content separately in pastry samples where fat and protein content vary independently.

Our experiments fulfilled these requirements so we were able to determine fat and protein content separately.

The correlation results presented here indicate that infrared reflectance measurements can be related to the compositional analysis of pastry products. The above-described studies lead to the conclusion that in the case of pastry products, the fat, protein, and water content can be predicted simultaneously with a quick, accurate, and nondestructive instrumental method using near-infrared reflectance data measured at some characteristic wavelengths. These results were obtained exploring only two of many possible data treatments and using only the optical geometry and sample size dictated by the

spectrophotometer. The repeatability and reproducibility values indicate that performance could be improved by recording more replicates, and it is also possible that the performance could be improved with other data treatments.

We also developed an equation for egg content determination, although it is valid only for wheat flour of the same composition and taking one whole egg as a mixture of 34 g white of egg and 16 g yolk of egg. In our opinion, the question to declare the egg content of a pastry product is not a task for the metrologie; however, the experts in instrumentation can significantly contribute to the solution.

*

Thanks are due to Ms. Joyce SHAFFER of the USDA-BARC-West INSTRUMENTATION LABORATORY for her conscientious technical assistance in performing measurements and data processing.

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Addresses of the authors:

DR. KÁROLY J. KAFFKA — Central Food Research Institute, H-1022 Budapest, Herman Ottó út 15. Hungary

KARL H. NORRIS — USDA-BARC-West, Instrumentation Research Laboratory, Beltsville, Maryland 20705. USA

MS. MÁRIA ROSZA-KISS — Research Institute of Flour Milling and Baking Industries, H-1117 Budapest, Dombóvári út 5–7. Hungary

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# CHANGING TENDENCY OF UTILIZATION OF PRODUCTION FACTORS AND THEIR EFFICIENCY RELATIONS IN THE FOOD INDUSTRY

K. Kóbor and M. Szabó

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The three basic resources of production in the food industry are dealt with, namely live labour, fixed assets, and raw materials of agricultural origin, with a view to efficiency. Although it is difficult to analyse the utilization of certain production factors, a definite tendency can be laid down: The increase of live labour productivity by 2.4% per year during the period 1960–1978 was followed by a 15% decrease in the fixed assets productivity. The degree to which foods are processed increased, although this growth was hardly demonstrable in the specific price structure of food production.

In this situation it was necessary to substitute live labour for fixed assets to an increasing rate, but this substitution required specifically more and more fixed assets. The costs of substitution doubled between 1960 and 1975 and will quadruple till 1985. Consequently, decreasing returns on inputs are expected in the future.

The aim of this paper is to summarize the work performed within the framework of research topics studied at the Economic Research Institute of the Food Industry during the period 1977–79.

In addition to the above-mentioned aims, our investigations have been extended to the study of the degree of processing of agricultural raw materials, to the description and analysis of production factors, to prognostication, and to plan-consistency studies. In the course of the plan-consistency study, we examined the quantitative harmony between planned production results and production factors.

Our investigations have been extended to the whole of the food industry including its most important branches: the meat, poultry, dairy, canning, milling, vegetable-oil, sugar, wine and brewing industries. The analyses were performed on branch level during the periods 1960–77 and 1968–78.

It is considered important to define some of the concepts discussed below.

Efficiency is understood to mean the relation of inputs to the output on the social level. It cannot be completely quantified and it is not determined by economic aspects only. The ratio of the value of production and fixed assets, of manpower and the raw material quantity are called indices of fixed assets efficiency, labour productivity and the degree of processing, these are regarded as partial indices of efficiency. They express the utility of single production

factors. (However, these economic efficiency indicators are only approximations in reality to that of the suitable partial efficiency indicators because of the deformation of the price system.)

Some problems impeded the measuring of economic categories. The added value would be the most suitable for measuring the production results, but it significantly fluctuates as a function of changes in the price system, where the fixed or maximized purchase and selling prices are changed at different points of time.

In the food industry, parallel to the expansion of production, the added value decreased in the past ten years, moreover in certain branches such as the meat, poultry and dairy industries it was negative and this is contrary to logic.

While in kind only the production of those branches can be taken into account which produce homogeneous final products, we generally used the gross production value in our analyses which is not exposed to the same extent to the influence of price changes and which deforms upward because of the increasing cooperation between associated factories.

Among the indices which are characteristic of the use of fixed assets, the net fixed assets value does not express the actual productive capacity of the fixed assets because the assessment of the volume of amortization rates serves the reproduction time of fixed assets as well as the regulation of income formation. Instead of the gross fixed assets value, we counted with the original gross value of operating fixed assets, because this value contains the written off but still working fixed assets, too. With unchanged price data, only the age composition of fixed assets was screened off and the influence of technical development was taken into consideration.

Of the indices which are characteristic of manpower utilization, we used the number of completed working hours and the number of employees with regard to the fact that the statistical category: "worker" has not been in existence since the *Uniform System of Occupations* was introduced in 1975 in Hungary. In favour of the continuity of time series, the number of workers and working hours were counted back by estimation on the basis of the proportion in 1975 of workers to manual employees and of data of manual workers.

In order to avoid deformation in the analysis of the period 1968 to 1978, the manpower was expressed by the number of employees.

## 1. Production factors in the food industry

We consider manpower under fixed assets and the agricultural raw materials in many cases still determinative of the volume and quality of production, as basic factors of production.

*1.1. Manpower utilization*

Between 1968 and 1978 the number of employees steadily increased (by 2% annually), however at a lower rate than the 3.4% increase during the period 1960–68.

The main cause of increase was the demand on manpower of the new investments, satisfied mainly from resources outside the food industry.

In view of medium- and long-term plan targets the expected technical development and the possibilities for expanding the resources of labour force until the end of 1985 we may count with a slow increase in the number of employees, about 0.5% annually of which 0.4% is the share of manual workers. The number of employees increases only in those branches where new investments are to be expected thus there will be significant differences in the growth rate. Manpower in the preservation industry and, within the latter, in the canning industry will decrease, while in the refrigeration industry it will increase. Most of the branches cannot count on supplementary labour power, though in the meat and brewing industries, in accordance with the tendencies of earlier years, a significant increase in manpower is expected.

*1.2. Use of fixed assets*

The value of operating fixed assets increased by 4.5% annually between 1960 and 1968, more quickly than the average of the national economy (at the constant price in 1976). After 1968 the rate of increase doubled, it was 8.4%. An outstanding increase of fixed assets could be observed in the meat and dairy industries (13.8 and 13.1%, resp.).

In the food industry, too, the economy of fixed assets can be characterized by expansion. Old fixed assets were not exchanged for new ones parallel to increase in capacity. The rate of written off but still working fixed assets is high: it was 8% after 1973, but for example in the canning industry it reaches 14% and for machines and equipments approximates 25%.

*1.3. Use of raw materials of agricultural origin*

Raw material plays an important part in the formation of production costs, it generally exceeds half of their total amount. In branches which process animal products, the rate of raw materials in the production costs can be 80–90%.

The use of raw materials significantly increased in the past ten years. The average increase was 8% annually, but in the meat, poultry, dairy, sugar and vegetable oil industries it reached the 10% level, at current price (BALOGH *et al.*, 1978).

The agricultural land is a national property which cannot be extended, moreover we have to count with its decrease. Thus, increased attention has

to be paid to its better utilization. To achieve this, within the agricultural external production value we have to increase the proportion of those raw materials which can be processed by the food industry and we have to transform product structure to the advantage of goods, representing a higher degree of processing. This would, at the same time, mean an increase in regional productivity.

## 2. Efficiency of use of production factors

The gross production value of the food industry increased threefold between 1960 and 1978 (at current price). The increase in production was slower in the sixties but later it became faster (KÓBOR *et al.*, 1978).

### *2.1. Productivity of labour*

The increase of productivity in the food industry was even, but slower (2.4% annually) than the average of national economy (at the constant price 1976). At current prices the value would be 2.9%.

Of the branches examined, only the poultry, dairy and canning industries showed relatively continuous development, while in other branches there were problems with the raw material supply. The productivity steadily increased in the canning industry although it strongly depends on agricultural production. The rate of constant decrease of manpower in the canning industry exceeded the fluctuations in the quantity of raw materials to be processed. At the same time, the progress in mechanization enabled the fulfilment of plan tasks (Table 1).

The character of the outlined process was not influenced by other factors such as the change in product composition, or the structure of the work force, *etc.*

### *2.2. Efficiency of fixed assets*

The index of fixed assets efficiency decreased by 15% in the past 18 years (at current price). But within this period two stages can be distinguished. While efficiency generally increased in the sixties, later it decreased variously in the individual branches. At that time the growth rate of the fixed assets value was double of the gross production value: 8.4 and 4.5%, resp. (at the constant price in 1976).

The fixed assets efficiency decreased steadily year by year. There was a growth only in 1977 after the extremely bad year of 1976. Of the branches examined, efficiency improved only in the poultry industry (Table 2).

The causes of decreasing efficiency were as follows:

– during the intensive periods of economic development the supplantation of live labour by fixed assets is generally accompanied by a

Table 1

*Development of production value for one employee in the food industry*
(at current prices)

1000 Forints per head

| Branch | 1960 | 1965 | 1968 | 1970 | 1975 | 1978 |
|---|---|---|---|---|---|---|
| Meat industry | 1082 | 1319 | 1034 | 991 | 962 | 962 |
| Poultry and egg processing industry | 225 | 343 | 481 | 550 | 587 | 703 |
| Dairy industry | 378 | 539 | 694 | 748 | 805 | 1124 |
| Canning industry | 119 | 186 | 238 | 256 | 343 | 451 |
| Milling industry | 735 | 1068 | 1025 | 944 | 918 | 983 |
| Sugar industry | 462 | 577 | 541 | 361 | 502 | 732 |
| Vegetable oil industry | 728 | 973 | 954 | 995 | 1347 | 1750 |
| Brewing industry | 252 | 308 | 325 | 328 | 394 | 461 |
| Food industry | 354 | 489 | 528 | 521 | 590 | 702 |

(Source of information: Élelmiszeripari Adattár 1967, 1972, 1977; MÉM STAGEK data-service)

Table 2

*Development of fixed assets efficiency in the food industry, at current prices*
(gross production value on unit of operating fixed assets value)

Forints per forint

| Branch | 1960 | 1965 | 1970 | 1975 | 1978 |
|---|---|---|---|---|---|
| Meat industry | 7.00 | 7.81 | 7.31 | 4.93 | 3.15 |
| Poultry and egg processing industry | 3.44 | 3.99 | 5.04 | 4.59 | 4.65 |
| Dairy industry | 5.92 | 5.22 | 4.41 | 2.70 | 2.43 |
| Canning industry | 1.70 | 1.88 | 1.56 | 1.57 | 1.42 |
| Milling industry | 2.90 | 4.21 | 3.33 | 2.40 | 0.98 |
| Sugar industry | 1.34 | 1.37 | 0.66 | 0.73 | 0.89 |
| Vegetable oil industry | 3.63 | 4.04 | 2.90 | 2.97 | 3.20 |
| Brewing industry | 1.05 | 1.12 | 0.87 | 0.76 | 0.78 |
| Food industry | 2.46 | 3.04 | 2.57 | 3.15 | 2.10 |

(Source of information: Élelmiszeripari Adattár 1967, 1972, 1977; Statisztikai Évkönyv of the years of 1960–1978)

decrease in efficiency, which, in Hungary is enhanced further by the expensiveness of fixed assets in relation to live labour. (This period in the Hungarian food industry began in the early seventies);
- the increase of capital intensity can be connected also with the intensive period of economic development. This is proved by the incremental demand on assets quotient;*
- efficiency being taken into consideration at current prices, the faster rise in investment goods compared with the production prices may cause a downward deformation of the index.

We must not examine and qualify fixed assets efficiency in itself. The process of supplanting live labour by assets and the rising level of processing of raw materials have also to be taken into account. For instance, the improvement in product quality should be expressed in terms of the price, in practice, however, it is not always possible to achieve this either on the home markets or on the export ones. The steadiness of domestic prices can be justified if the quality of the product used to be lower than the standard in the past and the consumer was for some reason compelled to buy it. Development is a reduction of this ratio.

We have to distinguish between the indices of various branches depending on their requirement for assets. There is a high requirement for assets in the sugar, canning and brewing industries but it is low in the meat, poultry and dairy industries.

However, positive or at least inevitable causes may be behind the decrease of fixed assets efficiency. For instance, while in recent years the trend of fixed assets value increased exponentially in the milling industry, the value of production remained practically unchanged, because mostly the assets directly not involved in production were developed due to the shortage of storage capacity.

In certain branches the modernization of fixed assets advanced rapidly (meat and dairy industry) but it was not followed by a similar rise in production. In the meat industry the fixed assets efficiency declined to less than half of its previous level since 1968 (at the current price in 1976), but it is still much higher than the average of the food industry.

The meat industry probably stands before a qualitatively new period of development, in which the outstandingly high rate of fixed assets growth is going to decrease. At the same time the rate of decrease in efficiency may slacken.

It seems to be paradoxical: the setting of new machines into operation reduces efficiency. We must not leave out of consideration, however, that the

* The incremental demand on assets quotient is connected with the fixed assets value used for the production of unit gross or net production value.

process has been accompanied by the continuous and relative substitution of live labour for instruments, *i.e.* the increasing technical development of labour, and with investment in the meat industry the ratio of accessory infrastructural and environmental protective investments is high and sanitary requirements are strict because of the large share of export.

The minimum enlargement of the labour force would not be possible without better working conditions accompanying a higher technical level.

The situation in the canning industry is the reverse: the cause of the slight reduction in the fixed assets efficiency lays in the moderate development of fixed assets. On the other hand, fixed assets efficiency in the canning industry has always been lower than the average of the food industries, thus a significant reduction is hardly possible.

The development of fixed assets efficiency in certain branches is influenced by the quantity and quality of raw materials and by the proportion of yields. The relation between these factors is very complicated: *e.g.* the increase in the quantity of raw materials achieved by an exaggerated capacity utilization may lead to a decline in the proportion of output. In our macro-level study the influence of the raw material factor was measured by its quantity, knowing that thereby other factors are left out of consideration.

Fixed assets efficiency may change with the fluctuation of the quantity of raw materials; *e.g.* in the wine industry the highly efficient year of 1973 was followed by a 15% decrease in the index in 1974 of a very poor vintage.

In the past 10 years only 39% of investments served to replace out-of-date equipment, to maintain the level of productivity. Old equipment was not discarded, thus new equipment was not able to raise the average technical level and fixed assets efficiency. The situation in the meat, canning and brewing industries is especially bad. In these branches three-quarters of the investments have served the extension of production.

The disharmony between the capacity of agriculture and the food industry, and the chronic overdemand for certain products (*e.g.* beer) was responsible for this development. In the future, it will be essential to change the policy of fixed assets economy. An intensive fixed assets economy is urged also by the fact that most of the growth of production is exported, thus, the quality requirements are continuously increasing.

The past 10 years' trend of fixed assets value and production and the labour force situation forecast a further slight decline of fixed assets efficiency.

The formation of the gross production value was roughly followed by the use of agricultural raw materials. However, in the canning and sugar industries, the shortage of raw materials occasionally hindered growth.

### 3. Examination of the degree of processing of foods

The technology of food processing and packaging develops year by year. This results in a higher nutritional value, the possibility of a healthier nutrition, and the supply of needs at a higher level and perhaps the creation of new ones.

It is clear that the additional inputs, except those promoting marketing, serve the increase in use-value. It would be logical, if the use-value were proportional to the ratio of additional inputs to essential raw materials. The enterprise accountancy could give information on the degree of processing of individual products. However, the present recalculation activity does not permit of economic analysis. The information collected by the accountancy serve only cost-economic and clearing goals.

It has been shown that producers' prices in the meat industry are determined by the amount of meat included in the product.

Since the producers' prices do not represent the higher degree of processing, the quotient of producers' price and the cost of basic materials is unsuitable for measuring the degree of processing. Similarly, the deformations of the price system render impossible the approach on the basis of added value.

If the price does not represent the socially necessary inputs, we have to be satisfied with some evident differentiations in classification according to the degree of processing (*e.g.* salami represents an evidently higher degree of processing than the split pork), while we do not know anything about the extent of the difference. But this possibility is not satisfactory when the whole product structure has to be classified with a view to selectivity.

If any role is intended for enterprises in shaping of product structure, the accountancy system has to be approximated to requirements.

Since the degree of processing, that is the utilization index of agricultural raw materials, is regarded as the index of specific efficiency in the food industry, in the above described phenomena the contradiction between efficiency at the level of national economy and of enterprise profitability, manifests itself.

The macro-level analysis of the degree of being processed is not so difficult. In branch relation the shaping of the degree of processing is expressed also by the input coefficients of the input–output balance. The decrease in the direct input coefficient of agricultural origin signals the increased degree of processing. The value of this index was 0.55 in 1955, 0.50 in 1972 and 0.45 in 1976. This means hat one unit output of the food industry required less agricultural production. The tendency in the meat industry was the reverse: between 1972 and 1976, the index value increased from 0.78 to 0.80 and this is explained by the rise of purchase prices.

Far-reaching conclusions, however, cannot be drawn from the output coefficients, because

- their values are influenced also by the inner structural changes of the very heterogeneous food industry,
- their size is influenced by the current price relations of agriculture and food industry,
- they are available only for certain years and certain branches.

The index of the degree of being processed which is the quotient of the value of gross production and of the raw material, slowly increases at current prices. On the other hand, current price analysis demonstrates a decreasing degree of processing with great yearly fluctuations. The index value increased from 1960 but significantly decreased in 1965, then it remained at a more or less constant level. The difference can be traced back to the depressed character of food prices, and proves that the increase of the degree of processing at branch level is less and less represented by the present price system.

Because of the known problems, we tried to demonstrate the increase in the degree of processing by a natural measuring unit by referring the result of production (at constant price) to the use of material expressed in kind. (Only those branches which process relatively homogeneous raw materials could be examined this way.)

Of the eight branches of the food industry examined, the degree of processing increased only in the dairy, canning and wine industries, it was stagnant in the meat industry and the change was not appreciable in the poultry, vegetable oil, milling and sugar industries (Table 3).

Thus, it appears that the global degree of processing in the food industry is unambiguously measurable and evaluable either at constant or at current

Table 3

*The development of the degree of processing*[a] *in some food industry branches*

| Branch | 1966 | 1970 | 1975 | 1970/1966 (%) | 1975/1966 (%) |
|---|---|---|---|---|---|
| Meat indsutry (1 000 Forints per ton) | 36.1 | 42.7 | 35.8 | 118.3 | 99.2 |
| Poultry industry (1 000 Forints per ton) | 51.9 | 47.4 | 44.2 | 91.3 | 85.2 |
| Dairy industry (1 000 Forints per 1 000 litres) | 8.9 | 9.4 | 10.1 | 105.6 | 113.5 |
| Canning industry (1 000 Forints per ton) | 11.9 | 15.3 | 18.3 | 128.6 | 153.8 |
| Milling industry (1 000 Forints per ton) | 6.9 | 6.5 | 5.3 | 94.2 | 76.8 |
| Sugar industry (1 000 Forints per ton) | 1.9 | 1.9 | 1.7 | 100.0 | 89.5 |
| Vegetable oil ind. (1 000 Forints per ton) | 21.0 | 18.1 | 18.4 | 86.1 | 87.6 |
| Wine industry (1 000 Forints per hectolitre) | 1.4 | 1.9 | 2.1 | 135.7 | 150.0 |

[a] Gross production value for the use of a unit of agricultural raw material at current prices in 1976.

(Source of information: Élelmiszeripari Adattár 1967, 1972, 1977)

prices, or on the basis of natural comparisons. It is hard to prove the increase in the degree of processing from the aspect of economy.

At the same time, the development is apparent if considered from the aspect of the customer and of industrial technology. Undoubtedly, the degree of preparedness of foods increased, several new technological processes were introduced, the product development activity improved, though in international comparison the product structure of our food production is still obsolete.

From the consumer's point of view, the degree of processing is characterized by the degree of preparation for consumption. This feature is related to the regrouping of the surplus value on the raw material: the food industry takes upon itself more and more of the marketing and domestic tasks. In a system of English origin (BEKE & SCHUSTER, 1977) products fall into four categories according to their degree of processing. According to this concept, there is significant development in the poultry, dairy and especially in the refrigeration industry. PERSÖTZY (1979) classified these activities from the industrial-technological point of view on the basis of the raw materials' passing through each processing level.

By means of this method, weighting each level by the change of the ratio of individual activities we get a characteristic index for the degree of processing.

These examples demonstrate also that it is undesirable to increase the degree of processing at whatever cost, only if it is done profitably. It often occurs that foods processed to a lesser degree can be better converted into money.

For example on the Soviet meat market, the ratio of these products (beef cattle and bony meat) was 95% in 1977, while they received 96% of the sales receipts. In 1978 the ratio was 82 and 84%, respectively. Other signs, too, show that the appreciation of a higher degree of processing is not general on the world market.

In our opinion, increasing the degree of processing in the field of food production generally results in improving efficacy of the whole production process. This is supported by phenomena which are more or less independent of the current price and regulation system and can be outlined at least in the long run.

For instance:

- An increase in the degree of processing results in the better utilization of agricultural raw materials as well as in a rise in regional productivity.
- Both in agriculture and in the food industry, and within the food industry from level to level of processing, the specific development costs generally decrease together with the degree of processing.
- Because of shortage of raw materials we have to aim at the more extensive export of qualified labour.

## 4. Replaceability of production factors and their influence on production results

We tried to describe the process of production with production functions of different types. These functions related to different periods of time (KÓBOR *et al.*, 1978).

Because of the unfavourable but regular labour force situation, live labour has to be substituted at an increasing rate for fixed assets and substitution requires the use of a specifically increasing amount of assets.

Up to 1967, there was a more or less parallel increase in the labour force and the use of fixed assets (at current price, *i.e.* the expansion of labour resources actually exceeded the growth of assets). However, the technical level of labour force rapidly increased after 1967 (even at the constant price of 1976).

The cost of supplanting a worker by fixed assets increased two and a half times between 1960 and 1976. This cost will increase further by 80 per cent till 1985 (Table 4).

The indices of individual branches differ substantially. Substitution is less expensive in the canning and wine industries, while it is the most expensive in the dairy industry.

The capital costs seem to be very high in relation to the index of average technical level, but the results are supported by empirical data. The quotient of development costs and the maximum number of technical workers providing the growth of technical level is for some investments under planning and construction (substitution cost for 1960 = 100)[a] as follows:

| | |
|---|---|
| Baja Meat Processing Plant | 123 |
| Sárbogárd Reinforced Concrete Silo | 1 544 |
| Zalaegerszeg Fodder Mixer | 162 |
| Hajdúság Sugar Factory | 1 147 |

Calculated on the basis of data from the Planning Enterprise of the Food Industry.

It has to be taken into consideration that, in the period under survey, the rate of increase in the cost of fixed assets was high and, according to the current price prognostics, the same trend is to be expected in the future. The data do not necessarily mean that substitution is possible only at such a high price. The period described is characterized not only by substitution, but by the joint expansion of fixed assets. Our conclusions were drawn from this course of development. It is possible that, beside a development policy aimed at substitution, we get essentially different results after a qualitative change. Because of the high degree of aggregation, the results may be only considered

[a] The high values for significantly mechanized branches which require a considerable part of building arise from the character of technology. The supplementary infra-structural investments make the improvements more expensive.

Table 4

*Development of substitution costs of fixed assets for live labour*

| Year | The development of substitution costs (%) |
|---|---|
| 1960 | 100 |
| 1970 | 161 |
| 1975 | 229 |
| 1980 | 310 |
| 1985 (expected) | 440 |

averages; in certain cases it is possible to free labour only by reorganization, without any investment.

Analysing the relations of production factors, results of production and investments by correlation calculation, we could not demonstrate any correlation between the deviations from trends of raw materials and of gross production value in the whole of the food industry between 1960 and 1978.

Thus, a better agricultural year did not increase production above the planned value, and in the reverse case, it did not reduce it either. Because of lack of capacity, the surplus purchase increased the export of raw produce simultaneously. However, certain branches processed partly stored or not home-produced raw materials. Moreover, the equalizing influence of aggregation is also significant: weather unfavourable for a certain branch of agriculture can be advantageous for another one.

The connection as expected between the increase of production and of investments could not be demonstrated, and this indicates again the unsatisfactory efficiency of investments. The fluctuations in efficiency indices are related rather to the changes of production than to the utilization of factors.

Beside the inflexibility of labour force and of the quantity of fixed assets, a further cause may be the existence of certain unexploited reserves: the continuous and steady increase, as related to the base, was not significantly influenced by the use of production factors.

## 5. Possibilities of future development

The yields of additional inputs in certain factors justify that the conditions of increase become more difficult in the intensive period of development. The yields of additional fixed assets and raw material inputs decreased by 29 and 31%, resp., between 1960 and 1976. A unit of live labour growth, on the other hand, parallel to the scantiness in this factor is followed by twice as big growth in production as that at the beginning of the period (SZABÓ, 1979).

It relates to the joint and average decrease of productivity of factors as well as to the exhaustion of extensive reserves that their 1% joint increase, resp., goes together with an only 0.87% increase of production. The same index for the 1968–78 period is even less: 0.22%; it is decreasing in its tendency. Of the individual branches, the average productivity increases in the poultry, dairy, vegetable oil and brewing industries, decreases in the canning and milling industries, and remains at about the same level in the meat and wine industries (Table 5).

Table 5

*Dynamic efficiency indices of production factors*
(on the basis of the three-variable *Cobb-Douglas* production functions)

| Branch | Production growth for 1% live labour growth (%) | Production growth for 1% fixed assets growth (%) | Production growth for 1% fixed asset and live labour growth (%) |
|---|---|---|---|
| Meat industry | 0.91 | −0.01 | 0.90 |
| Poultry industry | 1.53 | 0.03 | 1.56 |
| Dairy industry | 1.97 | 0.12 | 2.09 |
| Canning industry | 0.09 | 0.74 | 0.83 |
| Milling industry | 0.55 | 0.17 | 0.72 |
| Vegetable oil industry | 1.11 | 0.79 | 1.90 |
| Wine industry | 0.09 | 0.93 | 1.02 |
| Brewing industry | 1.95 | −0.04 | 1.91 |
| Food industry total: | −0.47 | 0.69 | 0.22 |

(Calculated by the author based on data of Élelmiszeripari Adattár 1967, 1972, 1977; Statisztikai Évkönyv of the years of 1960–1968)

We tried to explain the increase in production from year to year on the basis of the yearly growth of fixed assets and of live labour, thereby implicitly inferring that the productivity of dispcsable production factors in a given year will not change in the next year and the change of joint productivity is exclusively due to the productivity of growth which is different from this (SZABÓ, 1979). We did not observe this connection in the whole of the food industry and in most of the individual branches, either, only in the dairy and poultry industry could the two-thirds of fluctuations be explained by the increase of live labour and fixed assets. The series of data are, however, too short for drawing conclusive results.

On the other hand, by introducing the raw material factor, 80% of the changes have became explainable, by both the raw material and fixed assets and the raw material and labour force combination. (We used the current price data of the period 1960–76.)

The results indicate that raw material, and primarily its volume, controlled the change of productivity in the past one and a half decade.

Examining the development courses of individual branches it appears that there would be a growth reserve in the increase of live labour (chiefly in the poultry and dairy industries). The increase of fixed assets would be accompanied by a significant increase of production, only in the wine industry. The reserves in the increase of factors seem to be exhausted in certain branches (canning and milling industry). Substitution is relatively cheap in the canning industry and perhaps live labour may be set free for other branches.

From the plan-consistency examinations we established that in the 6th Five-Year Plan-period presuming the same rate of increase in production, because of the exhaustion of labour force resources, the increase of fixed assets will become faster. For this reason, beside the reserves inherent in organization and in utilization of capacity, the growth rate of investments has to be increased. According to forecasts, heavily questionable, in view of the present tendencies in the live labour situation and in the increase of fixed assets, a modest increase is expected in certain branches and a decrease in the meat, poultry and brewing industries. If the increase of investments is not realized we have to reckon with the stagnation of production and with slackening of the growth rate of production.

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Address of the authors:

Dr. Kálmán Kóbor
Mr. Márton Szabó

Research Institute for Agricultural Economics
H-1032 Budapest, Zsil u. 3–5. Hungary

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# INVESTIGATION INTO THE INTERACTION OF DIFFERENT PROPERTIES IN THE COURSE OF SENSORY EVALUATION

## I. THE EFFECT OF COLOUR UPON THE EVALUATION OF TASTE IN FRUIT AND VEGETABLE PRODUCTS

GY. URBÁNYI

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According to common experience as well as to data in the literature the different properties of foods cannot be judged independently at sensory evaluation. An assay was made to establish whether the colour of food items affected the evaluation of taste.

Foodstuffs of homogeneous consistency, the colour of which could be modified with food colouring agents without affecting other properties, were selected as test material. The samples thus obtained were subjected to scoring. Scoring was carried out by both expert panels and unexperienced members. Results were evaluated by mathematical statistical methods. Control tests were carried out in the dark and under illumination by red light.

It was established that the evaluation of taste was influenced by colour differing from the natural. These samples obtained lower score than those of natural colour.

The influence of colour on the value of taste was stronger with panel members of no experience than with expert panelists.

The disturbing factors may be reduced or eliminated by setting up the panel from highly experienced members or by selecting appropriate conditions for the sensory evaluation (*e.g.* coloured illumination).

The appearance of food products frequently permits drawing conclusions as to other properties. Thus, for instance the colour of fruits is mostly indicative of their stage of maturity. Discoloration in fruits may show changes in their composition or even processes of spoilage. These facts influence the customer who is more inclined to buy goods of attractive appearance and natural, characteristic colour.

AMERINE and co-workers (1965) observed that of two kinds of margarine, white and tinted yellowish, otherwise of identical taste and odour, the white product could be sold only at a lower price.

It seems evident that sensory panel members are also influenced in judging other properties by the appearance and colour of food products.

MOIR observed as early as in 1936 that panel members made more mistakes in estimating the aroma of jellies if they were of unnatural colour. Similar observations were made by KANIG (1955) in the evaluation of colourless or unusually coloured syrups.

In sensory testing of wines PANGBORN and co-workers (1963) found that the estimation of sweetness was much more successful if the taste was accompanied by regular colour.

Similar conclusions may be drawn from the investigations of BENGTSSON and HELM (1946). They found that the taste of beer was more difficult to judge if it was presented in dark bowls instead of light ones.

Since data in the literature as well as common experience prove that the colour of the food under investigation may influence the estimation of taste, it was considered of interest to study this problem. The subject of the study was whether the differences in colour affected the estimation of taste in products otherwise of identical taste.

## 1. Materials and methods

### *1.1. Materials*

In selecting the materials for sensory evaluation care was taken to choose products of homogeneous consistency (puree or liquid) and suitable for colouring with food colouring agents without affecting perceptibly their other properties. Three kinds of puree (spinach, potato, apple) and apple juice were selected as test materials.

To modify the colour of these test materials, non-toxic, tasteless and odourless, water-soluble, red and rum brown colouring agents permitted for use in foods, were used in aqueous solution.

The "red" colouring matter contains *Amaranth* (E-123; CI 16 185), the "rum brown" pigment consists of a mixture of *Amaranth*, *Indigotin* (E-132; CI 73 015), *Brillant Black* (E-151; CI 28 444) and *Tartrazin* (E-102; CI 19 140).

### *1.2. Methods*

*1.2.1. Scoring.* Each test material was scored for taste and colour on a 10-point scale. The panels consisted of 7 to 10 members. Retasting of the samples was permitted.

*1.2.2. Duo-trio test.* These tests were carried out under illumination with red light. The aim was to discover whether panel members found any difference between samples of identical taste after blotting out the differences in colour.

Both tests were carried out by two panels one of them consisting of well trained experienced members, the other of unexperienced university students.

*1.2.3. Mathematical statistical evaluation of results.* The results of scoring tests were evaluated by *Kramer*'s method. The scores of each panel member were ranked individually, then the rank numbers belonging to each sample were added up and the rank sums thus obtained were compared with the limit value in *Kramer*'s tables (KRAMER, 1963). This permitted to establish

whether differences between samples were significant at the 95 or the 99% probability level.

The scores belonging to samples of identical taste were analysed also by pairs. The differences between the means of pairs were subjected to the $t$ test.

The duo-trio tests were evaluated by means of the tables given by AMERINE and co-workers (1965).

## 2. Results

The first test material used was spinach puree. This was prepared by the traditional kitchen technique. Differences in taste were introduced by adding garlic while colour differences were produced by the use of the red colouring agent. The evaluation of the results of scoring of the 6 samples thus obtained, of which 3 were of the same colour and two of the same taste, was carried out by *Kramer*'s method (Table 1).

Table 1

*Evaluation by ranking according to* Kramer *of the sensory scores of spinach puree samples*

| Properties studied | Samples | | | | | |
|---|---|---|---|---|---|---|
| | *A* | *B* | *C* | *D* | *E* | *F* |
| | Rank sums | | | | | |
| | Expert panel (n = 10) | | | | | |
| Colour | 20.5* | 21.5* | 21.0* | 50.5** | 44.5 | 52.0** |
| Taste | 22.0 | 31.5 | 32.0 | 35.0 | 45.0 | 44.5 |
| | Unexperienced panel (n = 10) | | | | | |
| Colour | 21.5* | 20.5* | 18.0** | 57.0** | 44.0 | 49.0* |
| Taste | 20.0 | 31.5 | 26.0 | 47.0 | 47.0 | 38.5 |
| | Joint result of the two panels (n = 20) | | | | | |
| Colour | 42.0** | 42.0** | 39.0** | 107.5** | 88.5* | 101.0** |
| Taste | 42.0** | 63.0 | 58.0 | 82.0 | 92.0 | 83.0 |

* = significant at $P \geq 95\%$ level
** = significant at $P \geq 99\%$ level
Samples: *A* = flavoured with garlic
*B* = basic sample
*C* = 1 : 1 mixture of samples A and B
*D* = sample A tinted with 0.3% red colouring agent
*E* = sample B tinted with 0.3% red colouring agent
*F* = sample C tinted with 0.3% red colouring agent

As is shown by the results, the differences of colour, achieved by adding colouring agents, are perceptible. Coloured samples had a brownish tint slightly differing from the natural. The taste of the coloured samples was found by both panels worse than that of the sample of identical taste but of natural colour.

The sensory value of the sample pairs of identical composition but of different colour was subjected to the $t$ test as well, for both panels (Table 2).

Table 2

*Analysis, by the* t *test, of the taste scores of tinted and non-tinted spinach puree samples of identical taste, based on the mean scores*

| Samples | $t$ value | Level of significance |
|---|---|---|
| | Expert panel | |
| $A : D$ | 2.91 | 98% |
| $B : E$ | 3.31 | 99% |
| $C : F$ | 2.67 | 95% |
| | Unexperienced panel | |
| $A : D$ | 6.02 | 99.9% |
| $B : E$ | 7.15 | 99.9% |
| $C : F$ | 3.44 | 99% |

For identification of samples see Table 1

As can be seen, panel members found a significant difference in the taste of samples of identical taste but different colour, probably due to the latter circumstance. The level of significance is higher for each pair of samples in the estimation of the unexperienced panel than in that of the expert panel.

The test materials of different colour and taste were achieved by the addition of salt and rum brown colouring agent to creamed potatoes. The evaluation according to KRAMER (1963) of the sensory tests is tabulated in Table 3.

Colour differences were unambiguously shown by the tests. As to the taste, the samples of natural colour were considered better. Samples containing the highest amount of salt were given the lowest score. The coloured member of this pair of samples received the lowest score from the trained panel at the 95% probability level and by the combined evaluation of both panels, as well.

Table 3

*Evaluation by ranking according to* Kramer *of the sensory scores of potato puree samples*

| Properties studied | Samples | | | | | |
|---|---|---|---|---|---|---|
| | *A* | *B* | *C* | *D* | *E* | *F* |
| | Rank sums | | | | | |
| | Expert panel (n = 8) | | | | | |
| Colour | 17.0 | 14.0* | 16.0* | 42.5 | 41.5 | 37.0 |
| Taste | 30.5 | 19.5 | 20.5 | 40.5* | 29.0 | 28.0 |
| | Unexperienced panel (n = 7) | | | | | |
| Colour | 15.5 | 12.5* | 14.0 | 36.0* | 35.5* | 33.5 |
| Taste | 26.0 | 24.0 | 17.5 | 28.5 | 26.5 | 24.5 |
| | Joint result of the two panels (n = 15) | | | | | |
| Colour | 32.5** | 26.5** | 30.0** | 78.5** | 77.0** | 70.5* |
| Taste | 56.5 | 43.5 | 38.0 | 69.0* | 55.5 | 52.5 |

* = significant at P $\geq$ 95% level
** = significant at P $\geq$ 99% level
Samples: *A* = flavoured with 0.66% NaCl
*B* = basic sample
*C* = flavoured with 0.33% NaCl
*D* = sample A tinted with 0.016% rum brown
*E* = sample B tinted with 0.016% rum brown
*F* = sample C tinted with 0.016% rum brown

The potato puree sample pairs of identical taste but different colour were also compared by the *t* test. The results of this analysis are given in Table 4.

No significant difference was found by the trained panel in the sample pair of original taste alone, while the level of significance was 90% in this case, too. Significant difference was found within all three sample pairs by the unexperienced panel.

The apple puree was also subjected to scoring in daylight and in the dark in order to find out whether the observed differences in taste were due to the colour differences. Results are summarized in Table 5.

The effect of colour is apparent. Samples *E* and *F* tinted with a greater amount of rum brown received the lowest score, while samples *A* and *B* of natural colour obtained high scores, when the colour of the samples was visible to the panelists.

The result of tests carried out in the dark was different from the results obtained in daylight. A definite tendency could not be discovered in the

Table 4

*Analysis, by the* t *test, of the taste scores of tinted and non-tinted potato puree samples of identical taste based on mean scores*

| Samples | *t* value | Level of significance |
|---|---|---|
| | Expert panel | |
| *A* : *D* | 3.06 | 98% |
| *B* : *E* | 2.00 | 90% |
| *C* : *F* | 2.75 | 95% |
| | Unexperienced panel | |
| *A* : *D* | 3.12 | 95% |
| *B* : *E* | 2.46 | 95% |
| *C* : *F* | 3.13 | 95% |

**For identification of samples see Table 3**

evaluation of the unexperienced panel. However, in both the evaluation of the expert panel and in the combined results, the samples containing citric acid were valued higher than the samples of original taste. Because of the influence of colour in judging the samples in daylight this differentiation did not become apparent. These experiments prove also that the colouring agents used were indeed tasteless. The differences in taste as observed among samples of identical taste in evaluations in the daylight were due solely to the influence of differences in colour.

The *t* test of these sample pairs of identical composition but of different colour showed that in daylight the differences found by the panel were significant, while in the dark no significant difference was found in any of the samples.

In the sensory evaluation of apple juice differences of taste were not induced. All the samples subjected to evaluation differed only in their colour. Red and rum brown colouring agents were used. The colour of samples tinted with red was strange, differing from the natural colour of apple juice. The samples tinted with rum brown were nearer to the natural colour and suggested some fault in the manufacturing process.

These samples were also subjected to the judgement of two panels and the test was repeated with samples of identical composition under identical conditions at a later date. The evaluation of the two series of tests by *Kramer*'s method is summarized in Table 6.

The sample of natural colour obtained the highest score for taste as well as for colour by both panels at both dates and the difference was found,

Table 5

*Evaluation by ranking according to* Kramer *of the sensory scores of apple puree samples*

| Properties studied | Samples | | | | | |
|---|---|---|---|---|---|---|
| | *A* | *B* | *C* | *D* | *E* | *F* |
| | Rank sums | | | | | |
| | Tested in daylight | | | | | |
| | Expert panel (n = 8) | | | | | |
| Colour | 15.0* | 9.5** | 29.5 | 26.5 | 46.0** | 41.5 |
| Taste | 22.5 | 20.0 | 33.0 | 21.0 | 35.0 | 36.5 |
| | Unexperienced panel (n = 9) | | | | | |
| Colour | 21.0 | 17.5* | 29.0 | 27.0 | 49.0** | 45.5* |
| Taste | 19.0 | 39.0 | 30.0 | 27.0 | 31.5 | 42.5 |
| | Joint result of the two panels (n = 17) | | | | | |
| Colour | 36.0** | 27.0** | 58.5 | 53.5 | 95.0** | 87.0** |
| Taste | 41.5 | 59.0 | 63.0 | 48.0 | 66.5 | 79.0* |
| | Tested in the dark | | | | | |
| | Expert panel (n = 8) | | | | | |
| Taste | 35.5 | 23.0 | 32.5 | 23.0 | 29.0 | 25.0 |
| | Unexperienced panel (n = 9) | | | | | |
| Taste | 30.5 | 33.5 | 31.5 | 32.5 | 35.0 | 26.0 |
| | Joint result of the two panels (n = 17) | | | | | |
| Taste | 66.0 | 56.5 | 64.0 | 55.5 | 64.0 | 51.0 |

* = significant at $P \geq 95\%$ level
** = significant at $P \geq 99\%$ level
Samples: *A* = basic sample
*B* = flavoured with 0.16% citric acid
*C* = basic sample tinted with 0.01% rum brown
*D* = sample *A* flavoured with 0.16% citric acid, tinted with 0.01% rum brown
*E* = sample *A* tinted with 0.03% rum brown
*F* = sample *A* flavoured with 0.16% citric acid, tinted with 0.03% rum brown

with one exception, significant. With all the other samples, the sequence in taste and in colour was found to be of similar tendency.

The scores assigned to the taste of samples tinted in different ways were compared by the *t* test with that of the original sample (Table 7). A significant

Table 6

*Evaluation by ranking according to* Kramer *of the sensory scores assigned to apple juice samples*

| Properties studied | Samples A | B | C | D | E |
|---|---|---|---|---|---|
| | Rank sums | | | | |
| | First test | | | | |
| | Expert panel (n = 8) | | | | |
| Colour | 34.5* | 34.5* | 26.0 | 17.0 | 8.0** |
| Taste | 24.0 | 28.0 | 29.0 | 20.0 | 19.0 |
| | Unexperienced panel (n = 8) | | | | |
| Colour | 32.5 | 25.5 | 29.0 | 18.5 | 14.5* |
| Taste | 36.0** | 28.5 | 24.0 | 20.5 | 11.0** |
| | Joint result of the two panels (n = 16) | | | | |
| Colour | 67.0** | 60.0 | 55.0 | 35.5 | 22.5** |
| Taste | 60.0 | 56.5 | 53.0 | 40.5 | 30.0** |
| | Second test | | | | |
| | Expert panel (n = 7) | | | | |
| Colour | 22.5 | 24.0 | 26.0 | 24.0 | 8.5** |
| Taste | 24.5 | 22.5 | 24.0 | 24.0 | 10.0** |
| | Unexperienced panel (n = 7) | | | | |
| Colour | 25.0 | 24.0 | 25.5 | 21.5 | 9.0** |
| Taste | 28.0 | 24.5 | 23.0 | 18.0 | 11.5* |
| | Joint result of the two panels (n = 14) | | | | |
| Colour | 47.5 | 48.0 | 51.5 | 45.5 | 17.5** |
| Taste | 52.5 | 47.0 | 47.0 | 42.0 | 21.5** |

* = significant at $P \geq 95\%$ level
** = significant at $P \geq 99\%$ level
Samples: *A* = tinted with 0.0088% red colouring agent
*B* = tinted with 0.0044% red colouring agent
*C* = tinted with 0.0055% rum brown
*D* = tinted with 0.00275% rum brown
*E* = basic sample

Table 7

*Analysis, by the* t *test, of the taste scores assigned to tinted and non-tinted apple juice samples of identical taste, based on mean values*

| Samples | *t* test | Level of significance |
|---|---|---|
| **Expert panel** | | |
| | First test | |
| *A* : *E* | 4.14 | 99% |
| *B* : *E* | 4.14 | 99% |
| *C* : *E* | 4.24 | 99% |
| *D* : *E* | 3.86 | 99% |
| | Second test | |
| *A* : *E* | 2.81 | 95% |
| *B* : *E* | 3.07 | 95% |
| *C* : *E* | 3.22 | 98% |
| *D* : *E* | 3.22 | 98% |
| **Unexperienced panel** | | |
| | First test | |
| *A* : *E* | 5.92 | 99.9% |
| *B* : *E* | 7.18 | 99.9% |
| *C* : *E* | 4.31 | 99% |
| *D* : *E* | 6.12 | 99.9% |
| | Second test | |
| *A* : *E* | 3.17 | 98% |
| *B* : *E* | 3.22 | 98% |
| *C* : *E* | 4.60 | 99% |
| *D* : *E* | 4.05 | 99% |

For identification of samples see Table 6

difference was observed between all the scores while there was no real difference in the taste of the samples.

To confirm that this observation was due solely to the effect of colour differences, the samples were compared in pairs by the duo-trio test under red light blotting out the colour differences. Significant difference could not be found at the level of 95% between either of the sample pairs (Table 8).

Table 8

*Evaluation by the duo-trio test in red light of the taste scores of apple juice samples*

| Samples | Number of tests | Number of correct judgements | Number of incorrect judgements |
|---|---|---|---|
| $A : E$ | 14 | 8 | 6 |
| $B : E$ | 14 | 9 | 5 |
| $C : E$ | 14 | 4 | 10 |
| $D : E$ | 14 | 5 | 9 |

For identification of samples see Table 6

## 3. Conclusions

The mathematical statistical evaluation of the sensory tests carried out by an expert and an unexperienced panel on four test materials permit of the following conclusions.

In sample series consisting of samples of different taste as well as of different colour (spinach, potato and apple purees) it was established:

– Both the taste and colour of samples the colour of which differed from the natural were scored lower than those of natural colour.

– The judgement of unexperienced panel members was more affected by colour than that of the expert panelists.

In series of samples where the taste was identical only the colour differed it was established:

– The taste of the samples of characteristic original colour was found significantly better than that of the samples of unnatural colour or indicating some trouble on the manufacturing line.

— When colour differences were masked, the taste of the same samples could not be differentiated.

— If the colour induced was not alien to the product, the evaluation of other properties (*e.g.* taste) was not much influenced.

— The colour differing from the natural affected the judgement of the unexperienced panel members more than that of the expert panel members.

Based on the above, it may be concluded that colour differing from the natural one affected the estimation of taste in scoring tests. This influence was more extensive with unexperienced panels than with expert ones. Thus, it seems necessary to form panels from well trained expert members. In institutions where sensory tests are carried out regularly, it is desirable to train the members.

The objective estimation of taste may be promoted by conditions which hinder or eliminate completely the comparison of colours. To achieve this, it is suggested to apply illumination which masks the colour differences, or the presentation of the samples in bowls which do not permit the observation of colour (such as dark bowls for liquid foods). Naturally, in such cases the colour of the samples should be tested at another occasion under appropriate conditions.

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Address of the author:

Dr. György URBÁNYI Department of Food Technology and Microbiology, University of Horticulture
H-1118 Budapest, Ménesi út 45. Hungary

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# INVESTIGATIONS ON THE POSSIBLE GENOTOXIC EFFECT OF IRRADIATED ONION POWDER BY MEANS OF PROPHAGE INDUCTION (INDUCTEST)

J. FARKAS and É. ANDRÁSSY

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Aqueous extracts and enzymic digests of onion powder, untreated, irradiated with 5.0 and 10.0 kGy gamma radiation doses under aerobic conditions were tested with lysogenic strains GY 5022 and GY 5027 of *Escherichia coli* K12, for their prophage λ inducing effect in the course of the genotoxicologic study carried out as a part of the wholesomeness testing of irradiated spices and condiments in the frame of the International Project in the Field of Food Irradiation (IFIP). The tests were carried out on onion powder stored for 3 months subsequent to radiation treatment. One μg of aflatoxin $B_1$ or 1 μg of streptozotocin per test was used as a positive control. The amounts of extracts and enzymic digests exposed to prophage induction test corresponded to about 55 mg and 22 mg, resp., of onion powder. While both aflatoxin and streptozotocin, known for their carcinogenic and mutagenic effect, exerted prophage induction, no statistically significant difference was observed between the frequency of spontaneous phage induction and that occurring in the presence of either untreated or irradiated onion powder. In tests carried out with thermally inducible λc 1857 test organisms (*Escherichia coli* K12 GY 5024 and GY 5029) onion powder, used in amounts as indicated above, did not damage either the prophage or the host organism in a way to affect the induced prophage propagating capacity of the cells.

The utilization of dried onion is on the rise in household usage, in the catering trade and in the food industries alike. Its microbial contamination, however, is frequently objectionable and the various components of the microbial flora may cause, depending on the character of use, different troubles (*e.g.* thermophilic spore former in the canning industry, psychrotolerant strains in refrigerated products or cold dishes, pectolytic bacteria in pickled products).

Fumigation with ethylene oxide is a frequently applied method of decontamination, however, particularly in the case of onion powder, it is wearisome, labour consuming and expensive (GALETTO *et al.*, 1979). Because of the presence of chemical residues (STIJVE *et al.*, 1976) and occupational health problems its application is more and more objected to.

A method appearing according to many tests suitable to replace fumigation and at the same time to be a more effective treatment, is treatment with ionizing radiation (VAJDI & PEREIRA, 1973; FARKAS, 1973; FARKAS *et al.*, 1973; KISS *et al.*, 1978; SZABAD & KISS, 1979). Its technological feasibility was proved in studies with onion powder too, and a dose requirement of 2 kGy was established, depending on the degree of microbial contamination and on

the decontamination requirement (FARKAS & EL-NAWAWY, 1973; DELKINOVA & DUPUY, 1973; SILBERSTEIN *et al.*, 1979a). Radiation doses sufficient to reduce cell count to the desired level cause chemical and sensory changes of a practically negligible degree (FARKAS & EL-NAWAWY, 1973; GALETTO *et al.*, 1979; SILBERSTEIN *et al.*, 1979b).

In judging the wholesomeness of irradiated onion powder one may relay on the mass of negative results obtained in testing irradiated food stuffs from this aspect. Among these results a great number relates to toxicological tests on irradiated onions (BARNA, 1979). However, most of them were carried out on onion irradiated at a low dose level. Therefore it appeared desirable to investigate the possible genotoxic effect of onion powder irradiated at a dose level necessary to reduce the cell count in the dried product.

The results obtained in *Salmonella*/mammalian microsome tests were published in an earlier paper (FARKAS & ANDRÁSSY, 1981b). The present paper is an account of the tests carried out by prophage induction *(Inductest)* (MOREAU *et al.*, 1976; MOREAU & DEVORET, 1977). The researchers who developed this method found a good correlation between carcinogenicity and prophage inducing capacity of many chemical agents as observed in rodents (HEINEMANN, 1971) and according to them the two phenomena can be traced back to the same biological mechanism (MOREAU & DEVORET, 1977). Thus, they suggest the method to be used as a sensitive test for the detection of potentially carcinogenic substances. The method is relatively simple and not too expensive and proved advantageous in testing the blood of rats fed on irradiated spices (FARKAS & ANDRÁSSY, 1981a). These experiments form a part of the research work on wholesomeness testing of irradiated spices and condiments carried out as a contribution to the International Project in the Field of Food Irradiation (IFIP) Karlsruhe, FRG.

## 1. Materials and methods

The onion powder used as test material was the product of the Kecskemét Canning Factory.

The onion powder was treated with 5.0 and 10.0 kGy radiation doses under aerobic conditions at room temperature with a RH–$\gamma$–30 type, self-shielded $^{60}$Co gamma radiation source of 7.0 kGy $h^{-1}$ dose rate.

The samples were stored for 3 months at 277 K (4 °C) prior to the phage induction tests. In the experiments lysogen strains GY 5022 envA uvr$^+$ ($\lambda$) and GY 5027 envA uvrB ($\lambda$) of *Escherichia coli* K12 were used as test organisms. To test the possible toxicity of the samples the wild type lysogens were replaced by strains GY 5024 envA uvr$^+$ ($\lambda$cI857) (ind$^-$) and GY 5029 envA uvrB ($\lambda$cI857) (ind$^-$) carrying thermally inducible prophage. The *Escherichia*

*coli* strain K12 GY 4015 served in both types of tests as indicator bacterium.

Aqueous extract and enzymic digest were prepared from the onion powder (PHILLIPS & ELIAS, 1978) according to the method as described in an earlier paper (FARKAS & ANDRÁSSY, 1981b).

The test method used was the one described by MOREAU and co-workers (1976) as *Inductest* III. A metabolising mixture prepared with the postmitochondrial fraction of rat liver homogenate was also used in every test. The prophage induction tests, 3 repetitions each, were carried out in 0.5 $cm^3$ of the aqueous extract and in 0.4 $cm^3$ of the enzymic digest. The aqueous extract per Petri dish corresponded to about 55 mg onion powder and the enzymic digest to about 22 mg onion powder.

Aflatoxin $B_1$ (1 or 10 $\mu$g per plate) and streptozotocin (1 or 10 $\mu$g per plate) in dimethyl sulfoxide solution (10 $\mu$l) were used as positive control. The former mycotoxin is a well-known carcinogen and its metabolites are potent mutagens and prophage inductors (MOREAU *et al.*, 1976; MOREAU & DEVORET, 1977). The antibiotic streptozotocin shows also carcinogenic, mutagenic and phage inducing effect, according to the related literature (HEINEMANN, 1971). The prophage inducing and mutagenic effect of both compounds has been observed also by the authors (FARKAS *et al.*, 1981; FARKAS & ANDRÁSSY, 1981a, b).

The authors attempted to find out also whether $H_2O_2$, which is a radiolysis product of aqueous solutions under aerobic conditions (SWALLOW, 1977) and according to TRUHAUT and SAINT-LEBE (1978) is formed in irradiated starch of 12–13 % moisture content at a concentration of 50 $\mu g \cdot g^{-1} \cdot kGy^{-1}$, is capable of phage induction under the given test conditions.

An equal volume of distilled water or dimethyl sulfoxide served as blind sample. The aliquots of the samples to be tested were mixed with 0.2 $cm^3$ suspension of lysogenic bacteria containing the metabolising mixture. The samples were then shaken and kept in a thermostat of 310 K (37 °C) for 20 min. After this induction period 3 $cm^3$ of soft agar and 0.3 $cm^3$ of indicator bacterium suspension were added to each sample and they were spread on GT-amp plates (MOREAU *et al.*, 1976). The plates were incubated for 18 h at 310 K (37 °C) and subsequently the infective centres were counted (plaques perceived in the lawn formed by the indicator bacterium). (Prophage induction becomes apparent by the increase in the number of infective centers as compared to that of spontaneous centers.)

In order to detect whether any of the samples damaged the prophage and the host bacterium and thereby affected the induced prophage propagating capacity of the cell the lysogens were replaced by λcI857 lysogens. In these experiments the mixing of the samples with the lysogen suspension containing the metabolising mixture and 20-min incubation was carried out at 305 K

(32 °C) instead of 310 K (37 °C) because at this temperature the prophage is not induced (ind$^-$) (MOREAU *et al.*, 1976). After mixing with the indicator system further incubation was carried out at 315 K (42 °C). At this temperature the repressor mutant λcI857 is inactivated and every one of the lysogenic cells turns into an infective center. Thus, the toxic effect of the samples becomes apparent in the reduction of the number of λcI857 infective centers (plaques).

All experiments were carried out in three replicates.

The "S9 mix" used in the experiments was prepared from the liver of rats treated with *Aroclor* 1254, according to AMES and co-workers (1975). The amount of the postmitochondrial fraction "S9" used was 25 μl per plate.

## 2. Results and conclusions

The results of the prophage induction tests are shown in Table 1 and those of the toxicity tests in Table 2.

To reduce the heterogeneity of the variances they were exposed to square root transformation and then to variance analysis. No significant difference

Table 1

*Results of prophage induction tests*

(Number of plaques per plate ± standard deviation)

| Sample | GY 5022 envA uvr+ (λ) | GY 5027 envA uvrB (λ) |
|---|---|---|
| Control (10 μl $Me_2SO$) | 12 ± 1 | 9 ± 1 |
| Aflatoxin $B_1$ (1 μg) | 33 ± 1*** | 807 ± 139*** |
| Streptozotocin (1 μg) | 23 ± 3*** | 36 ± 2*** |
| Control (0.1 cm$^3$ $H_2O_d$) | 12 ± 2 | 10 ± 2 |
| $H_2O_2$ (0.02 mM) | 14 ± 2 | 12 ± 2 |
| Control (0.5 cm$^3$ $H_2O_d$) | 14 ± 1 | 10 ± 2 |
| Onion powder (aqueous extracts) | | |
| 0 kGy | 16 ± 1 | 12 ± 3 |
| 5 kGy | 12 ± 2 | 11 ± 1 |
| 10 kGy | 14 ± 3 | 13 ± 2 |
| Control (0.4 cm$^3$ $Me_2SO$ 1 : 1) | 13 ± 3 | 8 ± 1 |
| Onion powder (enzymic digests) | | |
| 0 kGy | 14 ± 3 | 12 ± 1 |
| 5 kGy | 13 ± 3 | 11 ± 2 |
| 10 kGy | 13 ± 1 | 14 ± 1 |

The number per plate of lysogenic bacteria based on the optical density of the stock suspension, was about $10^3$

*** values very highly significantly differing from the control ($\alpha = 0.001$)

was found between the frequency of phage induction in the presence of either untreated or radiation treated onion powder and toxicity was not observed either.

In the presence of 1 $\mu$g aflatoxin $B_1$ the frequency of phage induction in strains GY 5022 and GY 5027 was found very highly significant ($\alpha = 0.001$). In the thermo-induction system this quantity of aflatoxin $B_1$ did not show toxicity manifesting itself in the phage-induction frequency with strain GY 5029, however, with strain GY 5024 the effect was positive and in the case of 10 $\mu$g it was positive for both strains. The frequency of infective centers as observed in the presence of aflatoxin $B_1$ was in good agreement with data in the literature (MOREAU *et al.*, 1976; MOREAU & DEVORET, 1977).

In the presence of streptozotocin in a quantity of 1 $\mu$g the number of infective centers increased, too, to a highly significant extent ($\alpha = 0.001$) for both test organisms. Toxicity of this compound was found only with strain GY 5024 and not with strain GY 5029.

Table 2

*Results of toxicity tests of the samples*
(Number of plaques per plate ± standard deviation)

| Sample | GY 5024 envA uvr+ ($\lambda$ cI857) | GY 5029 envA uvrB ($\lambda$ cI857) |
|---|---|---|
| Control (10 $\mu$l $Me_2SO$) | 1 260 ± 49 | 1 728 ± 64 |
| Aflatoxin $B_1$ (1 $\mu$g) | 1 000 ± 241* | 1 491 ± 193 |
| (10 $\mu$g) | 545 ± 24*** | 712 ± 8*** |
| Streptozotocin (1 $\mu$g) | 944 ± 96*** | 1 525 ± 184 |
| (10 $\mu$g) | 997 ± 138* | 1 531 ± 213 |
| Control (0.1 cm$^3$ $H_2O_d$) | 1 068 ± 40 | 1 619 ± 174 |
| $H_2O_2$ (0.02 mM) | 929 ± 46 | 1 488 ± 47 |
| Control (0.5 cm$^3$ $H_2O_d$) | 909 ± 76 | 1 680 ± 87 |
| Onion powder (aqueous extracts) | | |
| 0 kGy | 1 007 ± 171 | 1 509 ± 76 |
| 5 kGy | 1 057 ± 122 | 1 547 ± 345 |
| 10 kGy | 1 064 ± 134 | 1 531 ± 259 |
| Control (0.4 cm$^3$ $Me_2SO$ 1 : 1) | 1 097 ± 78 | 1 683 ± 169 |
| Onion powder (enzymic digest) | | |
| 0 kGy | 1 027 ± 114 | 1 461 ± 216 |
| 5 kGy | 1 003 ± 70 | 1 509 ± 40 |
| 10 kGy | 996 ± 113 | 1 634 ± 195 |

Number per plate of lysogenic bacteria, based on the optical density of the stock suspension, was about $10^3$

* values significantly differing from the control ($\alpha = 0.05$)

*** values very highly significantly differing from the control ($\alpha = 0.001$)

$H_2O_2$ showed neither phage inducing, nor toxic effect. It was observed, however, that the system, probably in consequence of the catalase activity of the metabolising mixture, decomposed the hydrogen peroxide.

The results obtained in the present study complement those obtained in *Salmonella*/mammalian microsome tests, in which mutagenic effect was not found either (FARKAS & ANDRÁSSY, 1981b). In experiments carried out with irradiated onion by other authors (SHILLINGER, 1973; ZAJTSEV *et al.*, 1973; MOHYUDDIN, 1975; HATTORI *et al.*, 1979) neither mutagenic, nor carcinogenic effect (IKEDA, 1975; ANON., 1977) could be observed.

*

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Address of the authors:

Dr. József FARKAS* } Central Food Research Institute
Ms. Éva ANDRÁSSY } H-1525 Budapest, Herman Ottó út 15. Hungary

* Present address: International Facility for Food Irradiation Technology (IFFIT)
6700 AB Wageningen-Hoog, P.O. Box 87.
The Netherlands

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# ATTEMPTS TO DETERMINE OIL, PROTEIN, WATER AND FIBER CONTENT IN SUNFLOWER SEEDS BY THE NIR TECHNIQUE

K. J. KAFFKA, K. H. NORRIS, J. PERÉDI and A. BALOGH

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Preliminary studies were made on sunflower seed grist samples with a very broad range of composition in order to establish how composition parameters, especially fat, protein, water, and fiber content, can be determined with reflection measurements in the near infrared wavelength region from 1000 to 2638.4 nm, and to estimate the accuracy in predicting the chemical constituents of sunflower seeds using the instrumental method mentioned.

A "one of a kind" computerized spectrophotometer built at USDA-BARC-West Instrumentation Research Laboratory, Beltsville, was used to study the sunflower seed grist samples specially prepared at the Research Institute for the Vegetable Oil and Detergent Industry, Budapest. Here these sunflower seed grist samples were also labelled with the composition parameters determined by traditional standard methods.

Near infrared reflectance factor (R) spectra were recorded for 90 sunflower samples. The spectra were then transformed to log (1/R) versus wavelength and to the second derivative of log (1/R) versus wavelength for correlation with compositional data. Linear stepwise regression techniques were used to determine the optimum wavelengths and other parameters for predicting each of the chemical constituents.

Also multi-term prediction equations–using only log (1/R) values at different wavelengths–were determined and described; the results were essentially the same as the above mentioned.

The possible application and limitation of the NIR technique as applied to sunflower seed was discussed. We concluded that NIR technique has the potential for use in rapid evaluation of sunflower seed quality.

The NIR technique applied to sunflower seed grist (averaging the measurements for three replicates) provides single-term prediction equations–using the ratio of second derivatives of log (1/R) measured at two characteristic wavelengths–giving correlation coefficients of 0.9988 for fat, 0.9932 for protein, 0.9980 for water, and 0.9912 for fiber. In a measurement time of less than 6 minutes for the three replicates of a sunflower seed grist sample, it was possible to determine fat content within a standard error of calibration of $\pm 0.643$ mass %, protein content within $\pm 0.654$ mass %, water content within $\pm 0.459$ mass %, and fiber content within $\pm 0.953$ mass %.

The increased demand in recent years for food constituent analysis and quality control has been met by adaptation of various techniques. One of these techniques that was found suitable for measuring a large number of constituents is based on the interaction of the sample with near infrared radiation. A beam transmitted or reflected by a sample contains information on the properties of the sample. A large number of food components have absorption peaks in the near infrared spectral region of 1 000–2 500 nm; therefore, this region is particularly useful for determining the composition of food products.

A beam transmitted or reflected by the sample is measured through the near infrared spectral range and the resulting spectra or their transformed curves are used to derive the composition of the sample.

BEN-GERA and NORRIS (1968) determined moisture content in soybeans by near infrared transmittance spectrophotometry.

HYMOWITZ and co-workers (1974) estimated protein and oil content in corn, soybean, and oat seed by near infrared reflection.

RINNE and co-workers (1975) predicted protein and oil content of soybeans by near infrared reflectance analysis.

NORRIS and co-workers (1976) predicted forage quality by near infrared reflectance spectroscopy.

NORRIS and BARNES (1976) used near infrared reflectance to predict nutritive value of feedstuffs.

MILLER and POMERANZ (1978) compared *Kjeldahl* and NIR procedures for interlaboratory and intralaboratory reproducibility of protein determination.

WILLIAMS and THOMPSON (1978) studied the influence of whole meal granularity (particle size distribution) on the accuracy of protein and water determination in wheat using the NIR technique.

Work has also progressed on the development of instruments for high-precision spectrophotometric measurements on food products.

MASSIE and NORRIS (1971) described a high-intensity spectrophotometer interfaced with a computer for food quality measurements.

ROSENTHAL (1973) mentioned in his report an instrument to determine moisture, oil, and protein content rapidly and accurately in grain and grain products by means of the NIR technique.

LANDA (1979) published a paper on food constituents analysis using a monochromator with high speed scan and high energy throughput.

On the basis of the above results, we decided to study the applicability of this NIR technique for determining the composition of the sunflower. So far, such measurements have not yet been reported in the scientific literature, although instrument manufacturers report that sunflower seeds can be analyzed with their NIR instruments.

The present study aims at finding the possibility for applying the NIR technique for the rapid determination of fat, protein, water, and fiber content in sunflower grist; and, at the same time, to predict accuracy for the different components, to choose the most suitable form of the regression equations, determine the parameters (characteristic wavelengths, coefficients, and constants) and last, but not least, to examine the achievable accuracy increase by studies on the transformed reflection spectra.

The results of these studies will hopefully enable us to develop a single-purpose instrument or to extend the usage of another one to be able to perform such measurements.

## 1. Materials and methods

The sunflower grist needed for elaboration of the NIR technique for a rapid, instrumental composition analysis was made up from five ingredients, namely:

granulated whole sunflower seed (*Krasnodar* variety),
granulated whole sunflower seed (*Ireg* variety),
hulled sunflower seed,
hull of the sunflower seed,
extracted sunflower seed (cake).

A rough grinding was first performed in a *Condux* type impeller mill, and afterward for obtaining a fine grain, a *Sraume* type cutting mill was used. Thorough mixing assured relatively homogeneous materials of which samples were blended to provide us with the 30 calibration samples.

Oil, protein, and fiber content were changed between extreme end-values turning up in industrial practice by adding different predetermined quantities of the five homogenized ingredients. Water was introduced with wetted, extracted sunflower seed (cake), and so it was possible to change the moisture content in the calibration samples.

Since the NIR technique is strictly correlative, the calibration samples are critically important, and the calibration samples should include all the variability in composition (range of constituent values), particle size, sample treatment, *etc.*, that might be encountered in any sample in the practice to be measured. Taking these all into consideration, the sunflower samples for calibration were carefully prepared at the Research Institute for the Vegetable Oil and Detergent Industry, Budapest. Here these sunflower grist samples were also labelled with the composition parameters determined by traditional standard methods.

The protein content determination for the thirty sunflower calibration samples was performed by using an automatic *Kjel-Foss* instrument type 162–10.

The oil content was determined on the basis of the corresponding Hungarian Standard (1976).

The dry matter content (moisture content) was determined on the basis of the corresponding Hungarian Standard (1977).

The raw-fiber content determination was performed on the basis of the corresponding Hungarian Standard (1975).

The relevant Hungarian Standard (1966) dealing generally with sunflower is No. 6830–66.

Each of the 30 different sunflower samples prepared at the Research Institute for the Vegetable Oil and Detergent Industry, Budapest, was packed into three cells; thus, we got a total of 90 samples to analyze and to use for

calibration. Having three replicates from the 30 original samples, we had the opportunity to reduce the errors by averaging the measurements.

We placed 10 $cm^3$ of sunflower grist sample into the sample holder (cell) of 38 mm diameter with a 1.2 mm thick special quartz window (type G. E. 124) on one side and pressed with a pressure of about 10 mPa from the other side. Illumination was done with a beam (the cross-section was not quite circular), the diameter of which varied between 15 to 20 mm at an incident angle of 0° through the quartz window (the illumination was perpendicular to the surface of the sample). Four lead sulfide detectors (sensors) were equally spaced around the incident beam to measure the radiation diffusely reflected by the sample at 45°.

The near infrared reflectance properties of sunflower samples were measured with a "one of a kind" computerized spectrophotometer built at USDA--BARC-West Instrumentation Research Laboratory, Beltsville. This instrument is built around a *Cary* Model 14 prism-grating monochromator with optics optimized for the near infrared. The monochromator is coupled to a digital computer for collection and analysis of data. The instrument is operated in a single-beam mode with a reference spectrum stored in the computer.

The monochromator was operated with slits at 2 mm giving an effective bandpass of 7 nm. The region scanned was 1000 to 2638.4 nm. The signal from the lead sulfide detectors were amplified, digitized, and fed to the digital computer. Reflectance data were collected every 0.2 nm with 256 digital conversions per point. Thus, we get 8 192 reflectance points for the whole wavelength region. The scanning speed was 20 nm per second, so the time period between two data collections was 20 ms; with one-half of the time for 256 readings during the "light period", and the other half to measure the "dark period" signal from the AC coupled amplifier in order to restore the absolute amplitude. To take a complete reflectance spectrum of a sample required about 165 seconds.

Ceramic material was used as a reference standard because its reflectance is the same for all wavelengths from 1 000 nm to 2 700 nm. The reflected signal from the ceramic standard was stored in the computer and used to divide the reflected signal from the sunflower samples. This gives a resultant curve of true diffuse directional-conical reflectance relative to the ceramic standard. (The term directional-conical reflectance is meant as a ratio of reflected flux collected through a conical solid angle to essentially collimated incident flux.) The computer corrects for the reflectance of the ceramic standard to give the real value of the diffuse directional-conical reflectance factor (R); the ratio of the flux actually reflected by a sample surface to that which would be reflected into the same reflected-beam geometry by an ideal (lossless), perfectly diffuse (lambertian) standard surface irradiated in exactly the same way as the sample. The diffuse directional-conical reflectance factor data were recorded at each measured reflectance point for each of the sunflower samples, and the

spectral data were stored on magnetic tapes for further processing. A new reference signal from the ceramic standard was stored in the computer about once each hour to minimize errors from long-term drift.

For data processing, the 8 192 point spectral curves were smoothed by a running average of 21 points at each point and shrunk to 1 024-point curves by choosing every eighth point and averaging it with adjacent points on either side. Compressed spectral curves were transformed to log (1/R) curves and recorded along with the compositional data for each sample. The transformation of the R spectral curves to log (1/R) proved useful because the log (1/R) function gives a linear correlation with the concentration of a given measured component. Plotting log (1/R) as a function of wavelength gives a spectral curve that is comparable to an absorption curve having peak readings at wavelengths that correspond to absorption bands in the sample. Preliminary studies indicated that performance could be improved by using the second derivative of the log (1/R) spectral curve rather than log (1/R). Therefore, all data were analyzed by use of the second derivative transformation of each wavelength point. The second derivative calculation was incorporated into the computer program for the linear regression analysis. A stepwise multiple-linear-regression program was used also to analyze the data and determine the optimum wavelengths and other important parameters for predicting fat, protein, fiber, and water content in sunflower samples.

Because changes in reflection are small, even from relatively large changes in composition, reflectance data must be recorded with high precision. For this reason, the resolution we used at the measurements was 0.0000305 log (1/R) unit (1 log (1/R) corresponds to 32 768 steps).

The definition of the standard error of calibration used in the discussion is:

$$\sqrt{\frac{\sum_{i=1}^{n}(Q_{si}-Q_{ci})^2}{n-1-p}}$$

where $Q_s$ is the respective quality parameter (dependent variable) determined by traditional standard methods, $Q_c$ is the same quality parameter computed from the regression equation, $n$ is the number of samples, $p$ is the number of independent variables.

The standard error of calibration is derived by comparing the laboratory values of one set of samples to the instrument values of that same set when creating the calibration equation. The standard error of prediction is obtained by comparing the laboratory values of a second set of samples to the instrument percent reading of that second set and verifying the existing calibration.

All measurements described were carried out in an air-conditioned laboratory at 296 K (+23 °C).

## 2. Results

The compositional data – namely oil, protein, water, and fiber content – of the 30 sunflower seed grist samples are summarized in Table 1.

We selected the log (1/R) spectra of four sunflower samples with high oil, with high protein, with high fiber content, and one with a normal composi-

Table 1

*The compositional data of the 30 sunflower seed samples*

| Sample No. | Protein content (mass %) | Oil content (mass %) | Water content (mass %) | Fiber content (mass %) |
|---|---|---|---|---|
| 1 | 19.70 | 54.55 | 3.09 | 3.81 |
| 2 | 18.75 | 50.65 | 3.88 | 8.84 |
| 3 | 18.40 | 32.70 | 8.10 | 25.68 |
| 4 | 17.80 | 45.93 | 3.66 | 11.59 |
| 5 | 20.15 | 40.93 | 4.06 | 12.61 |
| 6 | 22.50 | 36.68 | 4.66 | 12.93 |
| 7 | 24.70 | 33.13 | 5.45 | 13.67 |
| 8 | 27.05 | 28.46 | 6.45 | 13.16 |
| 9 | 29.15 | 24.09 | 6.38 | 12.76 |
| 10 | 18.15 | 49.69 | 3.52 | 11.46 |
| 11 | 16.55 | 44.97 | 4.09 | 16.46 |
| 12 | 15.35 | 40.00 | 4.49 | 20.82 |
| 13 | 14.70 | 37.80 | 4.71 | 23.06 |
| 14 | 12.35 | 29.30 | 5.56 | 30.02 |
| 15 | 19.90 | 30.94 | 7.89 | 25.82 |
| 16 | 20.50 | 29.26 | 7.66 | 25.30 |
| 17 | 21.70 | 27.58 | 8.27 | 24.37 |
| 18 | 22.70 | 27.14 | 8.95 | 23.03 |
| 19 | 23.85 | 25.02 | 8.73 | 21.62 |
| 20 | 24.80 | 22.89 | 8.11 | 21.09 |
| 21 | 19.15 | 41.78 | 5.88 | |
| 22 | 21.05 | 36.79 | 8.01 | |
| 23 | 22.50 | 32.76 | 10.62 | |
| 24 | 24.00 | 28.37 | 11.50 | |
| 25 | 25.50 | 23.97 | 15.46 | |
| 26 | 27.60 | 18.98 | 18.02 | |
| 27 | 29.80 | 14.93 | 20.88 | |
| 28 | 31.20 | 10.40 | 23.86 | |
| 29 | 33.35 | 5.76 | 25.83 | |
| 30 | 35.25 | 1.39 | 28.92 | |

tion, respectively, which are shown in Figs. 1 through 4. The second derivative curves of the corresponding log (1/R) spectra are also graphed in the same figures.

The figures illustrate that the second derivative of the log (1/R) spectra does show a much greater difference than log (1/R) between samples with different composition, sharpening the details in the curves.

The relationship between log (1/R) values and oil, protein, water, and fiber content of the 30 sunflower seed grist samples (90 spectra, three replicas of each sample) was determined by use of an iterative procedure repeated for up to three or four steps with the multiple regression analyses. The computer program determined the characteristic wavelengths and the coefficients from $K_0$ to $K_4$ that gave the best fit to the following equation for each measured component:

$$Q_{1i} = K_{0i} + K_{1i} V_{\lambda 1i} + K_{2i} V_{\lambda 2i} + K_{3i} V_{\lambda 3i} + K_{4i} V_{\lambda 4i}$$

where $Q_{1i}$ stands for the composition parameters (oil, protein, water, and fiber); $K_{0i}, \ldots K_{4i}$ are constants and coefficients, respectively; $\lambda_{1i}, \ldots \lambda_{4i}$ are characteristic wavelengths; $V_{\lambda 1i}, \ldots V_{\lambda 4i}$ are the log (1/R) values belonging to these characteristic wavelengths.

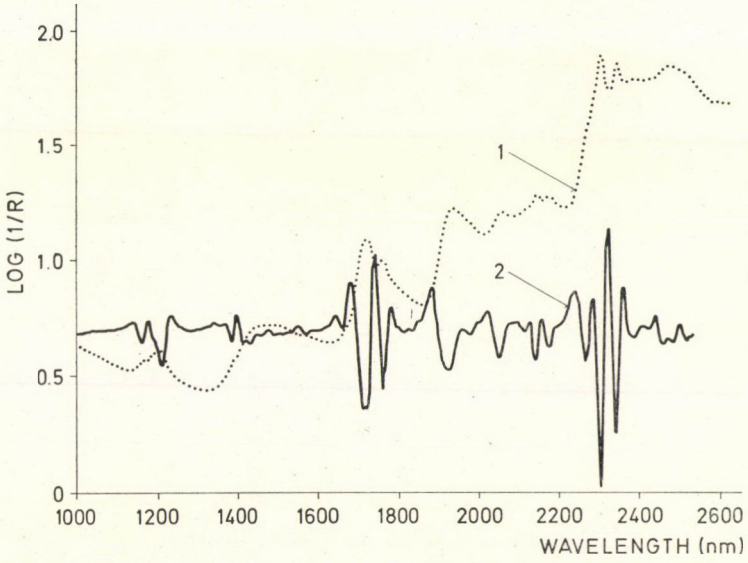


Fig. 1. The log (1/R) spectrum and the second derivative of sunflower seed grist sample No. 1 containing high percentage of oil. The measuring geometry: 0°/45°. Diameter of the illuminating beam: 20/15 mm. Spectral bandpass: 7 nm. Spectra were taken in 0.2 nm steps, compressed, then recorded in 1.6 nm steps. Vertical resolution 0.00003 log (1/R) unit. 1: Log (1/R) spectrum of sample No. 1; 2: Second derivative of the log (1/R) spectrum

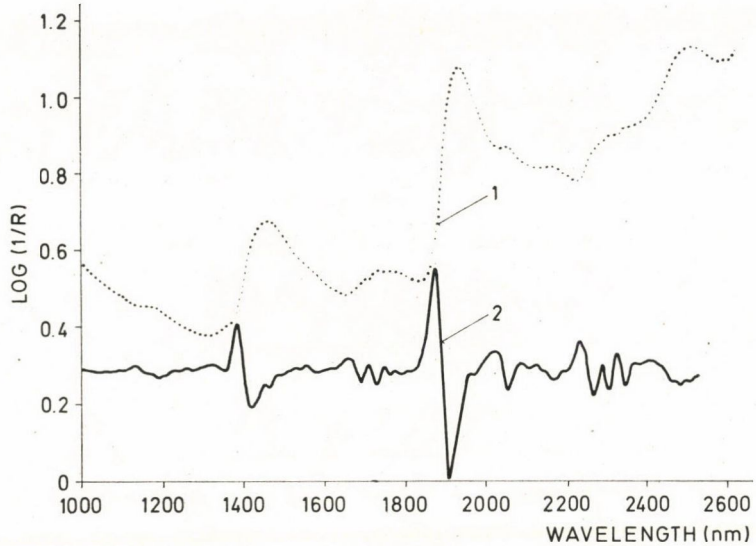


Fig. 2. The log (1/R) spectrum and the second derivative of sunflower seed grist sample No. 30 containing high percentage of protein. The measuring geometry: 0°/45°. Diameter of the illuminating beam: 20/15 mm. Spectral bandpass: 7 nm. Spectra were taken in 0.2 nm steps, compressed, then recorded in 1.6 nm steps. Vertical resolution 0.00003 log (1/R) unit. 1: Log (1/R) spectrum of sample No. 30; 2: Second derivative of the log (1/R) spectrum

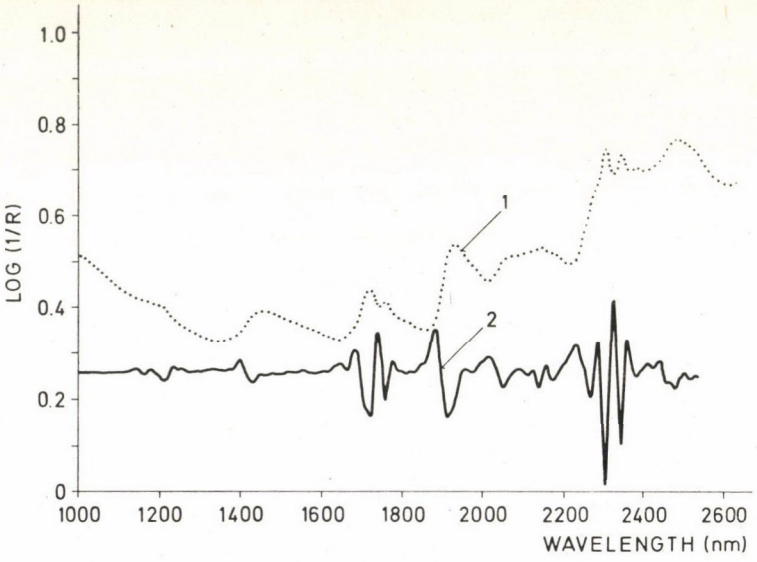


Fig. 3. The log (1/R) spectrum and the second derivative of sunflower seed grist sample No. 14 containing high percentage of fiber. The measuring geometry: 0°/45°. Diameter of the illuminating beam: 20/15 mm. Spectral bandpass: 7 nm. Spectra were taken in 0.2 nm steps, compressed, then recorded in 1.6 nm steps. Vertical resolution 0.00003 log (1/R) unit. 1: Log (1/R) spectrum of sample No. 14; 2: Second derivative of the log (1/R) spectrum

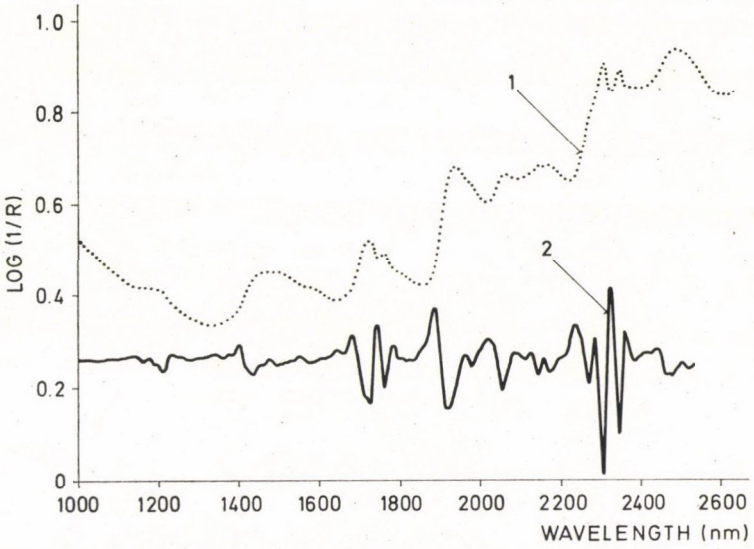


Fig. 4. The log (1/R) spectrum and the second derivative of sunflower seed grist sample No. 9 of an average composition. The measuring geometry: 0°/45°. Diameter of the illuminating beam: 20/15. Spectral bandpass: 7 nm. Spectra were taken in 0.2 nm steps, compressed, then recorded in 1.6 nm steps. Vertical resolution 0.00003 log (1/R) unit. 1: Log (1/R) spectrum of sample No. 9; 2: Second derivative of the log (1/R) spectrum

The results are summarized for oil, protein, water, and fiber content in Table 2, containing the characteristic wavelengths, the coefficients and constants of regression equations, as well as multiple correlation coefficients and standard errors of calibration.

We tried to find the relationship between composition parameters and log (1/R) values at other absorption bands of the above-mentioned components using the same equation form. The results were essentially the same in standard errors and multiple correlation coefficients.

Following this, we endeavored to increase accuracy by transforming the data to the second derivative of the log (1/R) spectra. The relationship between transformed spectral data and composition parameters were studied in the following form of equations:

$$Q_{2i} = K_{5i} + K_{6i} \frac{V''_{\lambda 5i}}{V''_{\lambda 6i}}$$

where $Q_{2i}$ stands for the composition parameters; $K_{5i}$ and $K_{6i}$ are coefficients; $V''_{\lambda 5i}$ and $V''_{\lambda 6i}$ are the values of the second derivatives of the log (1/R) spectra at $\lambda_{5i}$ and $\lambda_{6i}$ characteristic wavelengths.

Characteristic wavelengths, constants, and coefficients of these single-term regression equations, as well as standard errors of calibration and correlation coefficients, are summarized in Table 3.

Table 2

*Summary of linear regression analyses relating data from chemical analyses and log (1/R) values measured at three or four characteristic wavelengths for 30 sunflower seed grist samples, searching 1 000 to 2 500 nm*

Equation form: $Q_{1i} = K_{0i} + K_{1i}V_{\lambda 1i} + K_{2i}V_{\lambda 2i} + K_{3i}V_{\lambda 3i} + K_{4i}V_{\lambda 4i}$

| | For oil content | For protein content | For water content | For fiber content |
|---|---|---|---|---|
| Characteristic wavelength $\lambda_1$ (nm) | 2 312.0 | 1 924.8 | 1 867.2 | 1 752.0 |
| Characteristic wavelength $\lambda_2$ (nm) | 2 251.2 | 2 432.0 | 1 865.6 | 1 763.2 |
| Characteristic wavelength $\lambda_3$ (nm) | 2 092.8 | 1 998.4 | 1 681.6 | 1 755.2 |
| Characteristic wavelength $\lambda_4$ (nm) | — | 2 433.6 | — | 1 537.6 |
| Constant $K_0$ | 15.163 | 17.483 | 17.495 | 50.289 |
| Coefficient $K_1$ | 268.89 | −288.58 | 2 670.6 | 16 136.0 |
| Coefficient $K_2$ | −492.86 | −5 314.0 | −2471.3 | 16 762.0 |
| Coefficient $K_3$ | 177.87 | 388.07 | −234.33 | −32 593.0 |
| Coefficient $K_4$ | — | 5 263.6 | — | −308.42 |
| Standard error of calibration (mass %) | 0.892 | 1.000 | 0.639 | 0.767 |
| Multiple correlation coefficient | 0.9978 | 0.9856 | 0.9963 | 0.9953 |

Table 3

*Summary of linear regression analyses relating data from chemical analyses and values of the second derivative of log (1/R) curves at two characteristic wavelengths for 30 sunflower seed grist samples, searching 1 000 to 2 500 nm*

Equation form: $Q_{2i} = K_{5i} + K_{6i}\left(\frac{V''_{\lambda 5i}}{V''_{\lambda 6i}}\right)$

| | For oil content | For protein content | For water content | For fiber content |
|---|---|---|---|---|
| Characteristic wavelength $\lambda_5$ (nm) | 1 684.8 | 1 771.2 | 1 852.8 | 1 548.8 |
| Characteristic wavelength $\lambda_6$ (nm) | 2 012.8 | 1 667.2 | 1 643.2 | 1 867.2 |
| Constant $K_5$ | 6.6617 | 40.387 | −3.6030 | 68.339 |
| Coefficient $K_6$ | 176.32 | 21.253 | 8.2419 | −1 237.1 |
| Segment (wvl. range for averaging, nm) | 4.8 | 6.4 | 1.6 | 1.6 |
| Gap for numerator (nm) | 9.6 | 25.6 | 28.8 | 32 |
| Gap for denominator (nm) | 38.4 | 19.2 | 32.0 | 40 |
| Standard error of calibration (mass %) | 0.643 | 0.654 | 0.459 | 0.953 |
| Correlation coefficient | 0.9988 | 0.9932 | 0.9980 | 0.9912 |

In this table, the gaps for numerator and denominator, wavelengths, as well as the segments, are listed. The gaps are the distances among the three wavelengths used for producing the second derivative, while segment is the wavelength region in which the measured spectral data are averaged.

The oil, protein, water, and fiber have absorption bands in several regions of the near infrared, so we tested selected regions getting similar results.

Table 4

*Reproducibility and repeatability for sunflower seed grist samples expressed as the average of the standard deviations of the predicted data for oil, protein, water, and fiber using multi-term log (1/R) (1) and single-term second derivative (2) forms of equations*

| | Reproducibility average S. E. (mass %) | Repeatability average S. E. (mass %) |
|---|---|---|
| $Q_1$ FAT | 0.830 | 0.086 |
| $Q_2$ FAT | 0.515 | 0.105 |
| $Q_1$ PROTEIN | 1.534 | 0.886 |
| $Q_2$ PROTEIN | 0.447 | 0.084 |
| $Q_1$ WATER | 0.450 | 0.094 |
| $Q_2$ WATER | 0.276 | 0.026 |
| $Q_1$ FIBER | 1.625 | 1.028 |
| $Q_2$ FIBER | 1.014 | 0.834 |

The results for oil, protein, water, and fiber determination using log (1/R) values and the second derivative ratio—shown in Table 2 and Table 3—are graphed in Figs. 5 through 12.

The reproducibility was determined by comparing the spectra from the three replicas of the original thirty samples. Reproducibility was characterized by the average of the standard deviations of the predicted data of the values which can be seen for the different components and different equation forms in Table 4.

The repeatability was determined by recording ten spectral scans on each of three selected samples without changing the position of the samples. The selected samples were those containing extremely high percents of oil protein, and fiber, resp., (Sample No. 2, No. 28, and No. 14). The repeatability was characterized by the average of the standard deviations of the predicted data of ten scans for the three samples. The repeatability data for the different components and different equation forms are shown in Table 4.

We would like to note that the data in Table 4 characterize one single scan on sunflower seed grist, while the data in Table 2 and Table 3 characterize the average of the scans on three replicas of the same original samples. The

fiber results in these tables represent data from only 20 of the 30 samples (Table 1.) because the chemical fiber analyses were not available on the other 10 samples.

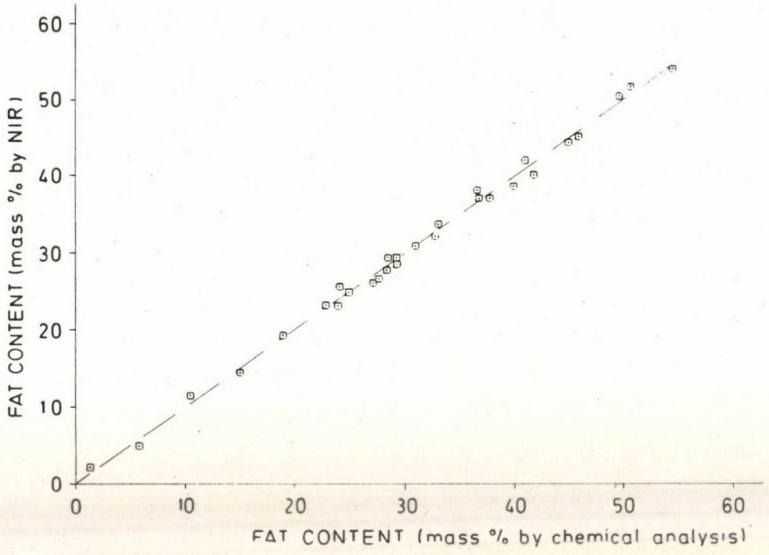


Fig. 5. Relationship between oil content determined by traditional chemical analysis and predicted value from multiple regression log (1/R) data at three wavelengths

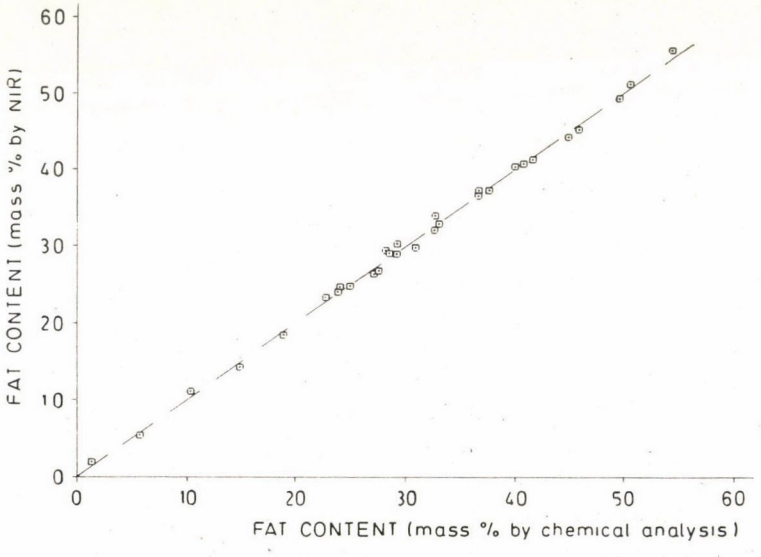


Fig. 6. Relationship between oil content determined by traditional chemical analysis and predicted value from linear regression of the ratio of the second derivatives of the log (1/R) curves at two wavelengths

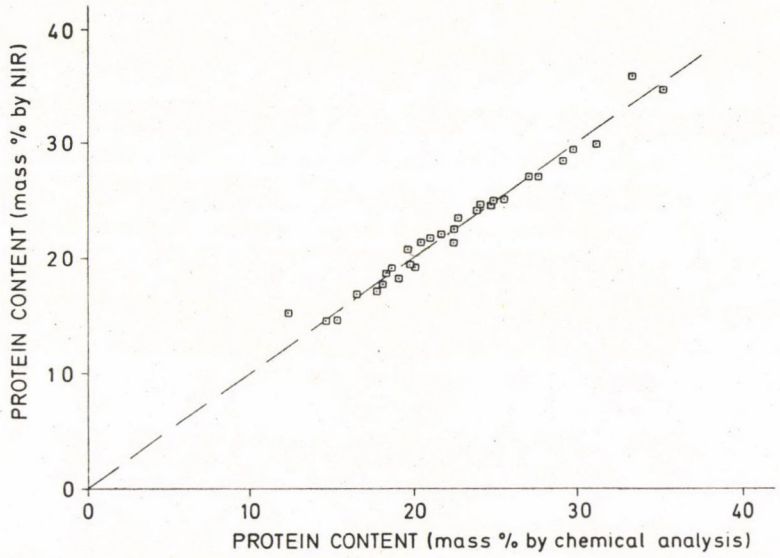


Fig. 7. Relationship between protein content determined by traditional chemical analysis and predicted value from multiple regression log (1/R) data at four wavelengths

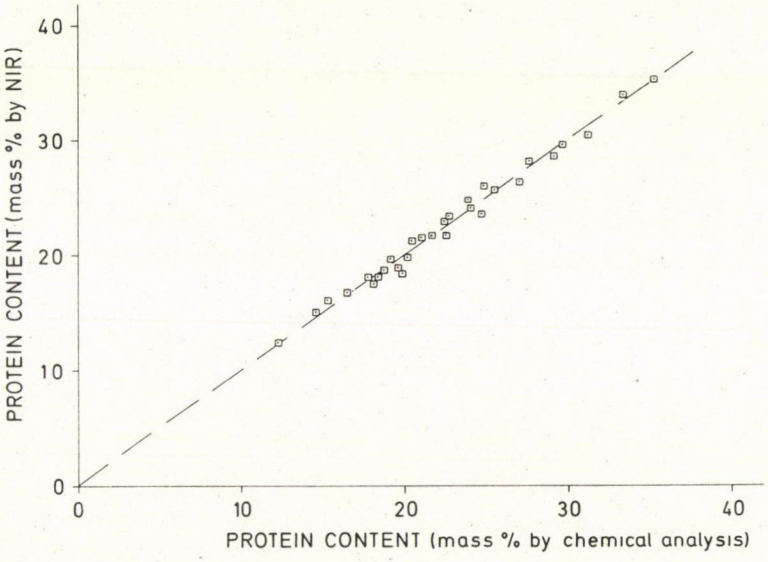


Fig. 8. Relationship between protein content determined by traditional chemical analysis and predicted value from linear regression of the ratio of the second derivatives of the log (1/R) curves at two wavelengths

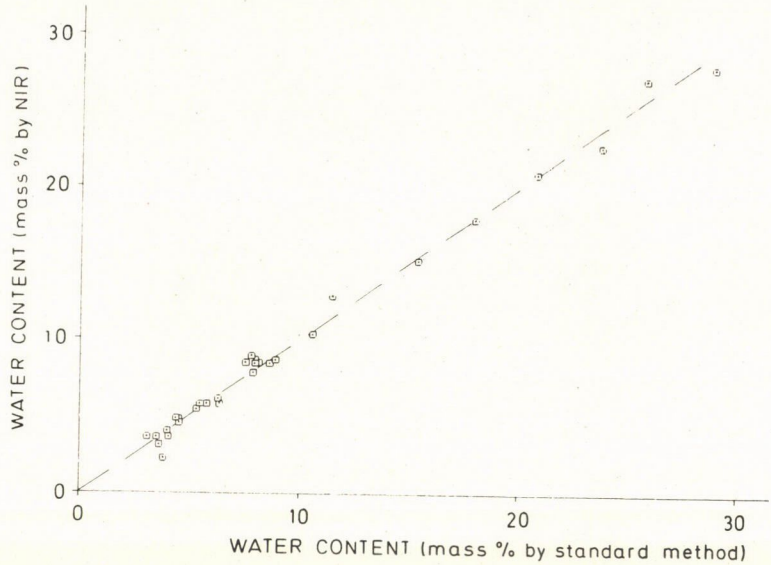


Fig. 9. Relationship between water content determined by traditional chemical analysis and predicted value from multiple regression log (1/R) data at four wavelengths

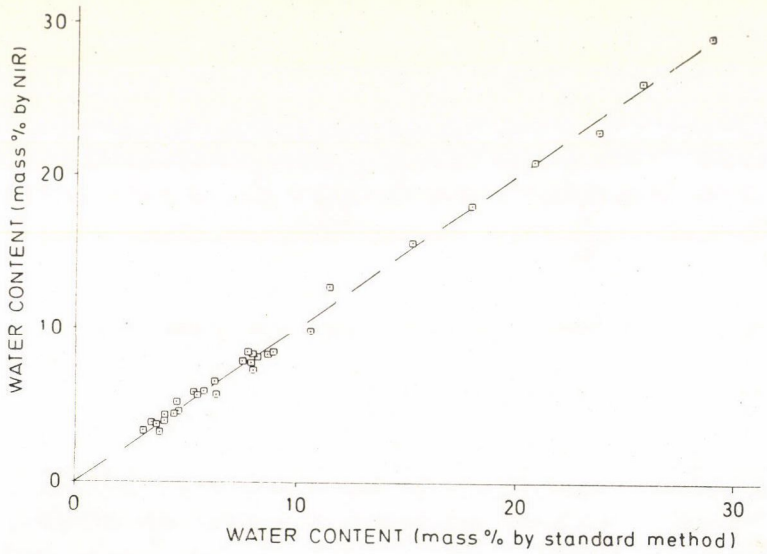


Fig. 10. Relationship between water content determined by traditional chemical analysis and predicted value from linear regression of the ratio of the second derivatives of the log (1/R) curves at two wavelengths

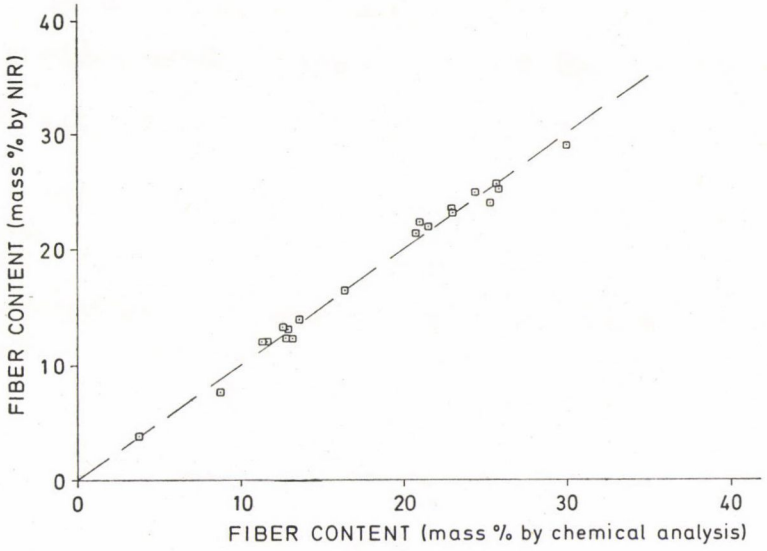


Fig. 11. Relationship between fiber content determined by traditional chemical analysis and predicted value from multiple regression log (1/R) data at four wavelengths

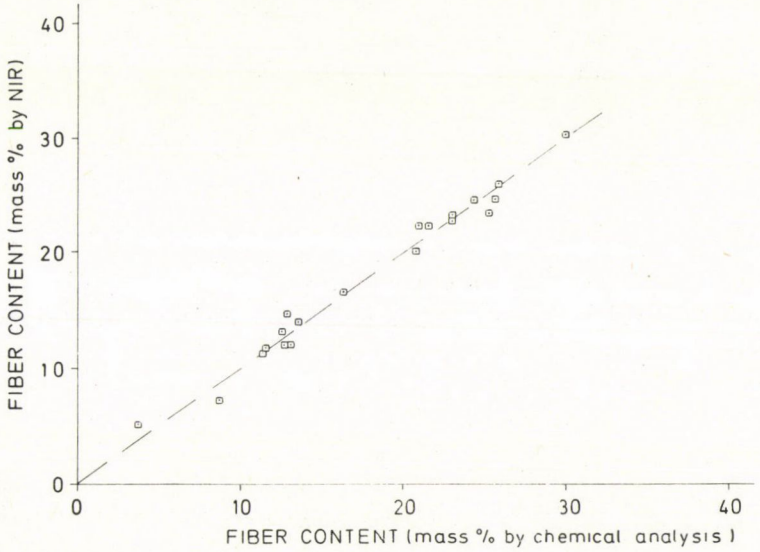


Fig. 12. Relationship between fiber content determined by traditional chemical analysis and predicted value from linear regression of the ratio of the second derivatives of the log (1/R) data at four wavelengths

## 3. Conclusions

As can be seen in Table 1, the oil content in our sunflower seed grist samples has a range from 1.39 to 54.55 mass %. The range of protein content is from 12.35 to 35.25 mass %. For water, the range is from 3.09 to 28.92 mass %. The range of fiber content is from 3.81 to 30.20 mass %.

Although the range of composition was extremely broad for all the investigated components, good results were obtained for oil, protein, water, and fiber content of sunflower seed grist samples using the NIR technique. The particle size distribution of the samples and its effect was not tested, but it was easy to establish that the range of particle size was also very broad in spite of the fact that the samples were prepared using the same mechanical treatment.

In all cases, no samples were omitted for the calculations, although the results could have been improved by omitting samples with large error.

Our experiments showed satisfactory accuracy for multi-term linear equations containing the log (1/R) values measured at different characteristic wavelengths; however, far better accuracy was obtained with the transformation of log (1/R) spectra; namely, by using the second derivatives of the spectra. In this case, we actually used a single-term equation where the value of the second derivative of the log (1/R) spectra at the first characteristic wavelength was divided by the value of the second derivative of the log (1/R) spectra at the second characteristic wavelength.

The correlation results presented here indicate that infrared reflectance measurements can be related to the compositional analysis of sunflower seeds. These calibration results have not been tested against unknown samples, but the very high correlation coefficients indicate that it should be possible to predict oil, protein, fiber, and moisture content with high accuracy using near infrared reflectance techniques.

These results were obtained by exploring only two of many possible data treatments and using only the optical geometry and sample size dictated by the spectrophotometer. The reproducibility and repeatability values indicate that performance could be significantly improved by recording more replicates, by increasing the diameter and intensity of the illuminating beam, and it is also possible that the performance could be improved by other data treatments.

*

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Addresses of the authors:

Dr. Károly J. KAFFKA — Central Food Research Institute, H-1022 Budapest, Herman Ottó út 15., Hungary

Karl H. NORRIS — USDA-BARC-West, Instrumentation Research Laboratory, Beltsville, Maryland 20705, USA

Dr. József PERÉDI, Ms. Antonia BALOGH — Research Institute for the Vegetable Oil and Detergent Industry, H-1106 Budapest, Maglódi út 6., Hungary

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# ATTEMPTS TO DETERMINE FAT, PROTEIN AND CARBOHYDRATE CONTENT IN COCOA POWDER BY THE NIR TECHNIQUE

KAFFKA, K. J., NORRIS, K. H., KULCSÁR, F. and DRASKOVITS, I.

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Preliminary studies were performed on cocoa powder samples with different, predetermined compositional values in order to establish how composition parameters, especially fat, protein, and carbohydrate content, can be determined with reflectance measurements in the near infrared wavelength region from 1 000 to 2 638.4 nm, and to estimate the accuracy in predicting the chemical constituents of cocoa powder using the instrumental method mentioned.

A "one of a kind" computerized spectrophotometer built at USDA-BARC-West Instrumentation Research Laboratory, Beltsville, was used to study the cocoa powder samples specially prepared at the Centre of Food Control and Analysis of the Ministry of Agriculture and Food, Budapest. Here these cocoa powder samples were also labelled with the composition parameters determined by traditional standard methods.

Near infrared reflectance factor (R) spectra were recorded for 60 cocoa powder samples. The spectra were then transformed to log (1/R) *versus* wavelength and to the second derivative of log (1/R) *versus* wavelength for correlation with compositional data. Linear stepwise regression techniques were used to determine the optimum wavelength and other parameters for predicting each of the chemical constituents.

The NIR technique applied to cocoa powder (averaging the measurements for three replicates) provides single term prediction equations—using the ratio of second derivatives of log (1/R) measured at two characteristic wavelengths—giving correlation coefficients of 0.999 for fat, 0.982 for protein, and 0.994 for carbohydrate. In a measurement time of less then 6 minutes for the three replicates of a cocoa powder sample, it was possible to determine fat content within a standard error of calibration of $\pm 0.134$ mass %, protein content within $\pm 0.186$ mass %, and carbohydrate content within $\pm 0.223$ mass %.

The possible application and limitation of the NIR technique as applied to cocoa powder are discussed. The NIR technique has the potential to be used in rapid evaluation of cocoa powder quality.

The increased demand in recent years for food constituent analysis and quality control has been met by adaptation of various techniques. One of these techniques that was found suitable for measuring a large number of constituents is based on the interaction of the sample with near infrared radiation. A beam transmitted or reflected by a sample contains information on the properties of the sample. A large number of food components have absorption peaks in the near infrared spectral region of 1 000–2 500 nm; therefore, this region is particularly useful for determining the composition of food products. A beam transmitted or reflected by the sample is measured through the near infrared spectral range and the resulting spectra or their transformed curves are used to derive the composition of the sample.

The demands for constant and improved quality of food products and especially of cocoa products requires the development of instruments for the rapid determination of quality parameters.

Fat, protein, and carbohydrate content are the most significant quality parameters of cocoa powder. The analytical methods for determining the above parameters are described in the respective Hungarian Standard (1975a). Accurate analysis for fat, protein, and carbohydrate in cocoa powder by traditional methodology is a slow process requiring special chemicals and several hours before test results are available. The time required for these chemical analyses is too long for automatic control of production. On the other hand, the time and working power demands also hinder the application of these methods for the control of quality of cocoa products in the shops. The time required for a determination using near infrared instrumentation is approximately two minutes, which is far more favourable than the wet chemistry method; and the measurement can be done simultaneously for fat, protein and carbohydrate.

HYMOWITZ and co-workers (1974) estimated protein and oil content in corn, soybean, and oat seed by near infrared reflection.

RINNE and co-workers (1975) predicted protein and oil content of soybeans by near infrared reflectance analysis.

NORRIS and co-workers (1976) predicted forage quality (crude protein, acid detergent fiber, neutral detergent fiber, dry matter, *etc.* content) by near infrared reflectance spectroscopy.

NORRIS and BARNES (1976) used near infrared reflectance to predict nutritive value of feedstuffs. In this paper cornstarch, soy protein, and cellulose spectra are demonstrated and compared.

MILLER and POMERANZ (1978) compared *Kjeldahl* and NIR procedures for interlaboratory and intralaboratory reproducibility of protein determination.

MINER and co-workers (1980) in a manuscript predicted fat and moisture content in different types of chocolate, cocoa powder, dust and shell, liquor, raw beans, and roasted beans.

Work has also progressed on the development of instruments for high-precision spectrophotometric measurements on food products.

MASSIE and NORRIS (1971) described a high-intensity spectrophotometer interfaced with a computer for food quality measurements.

ROSENTHAL (1973) mentioned in his report an instrument for the determination of moisture, oil, and protein content rapidly and accurately in grain and grain products by means of the NIR technique.

LANDA (1979) published a paper on food constituents analysis using a monochromator with high speed scan and high energy throughput.

On the basis of the above results, we decided to study the applicability of this NIR technique for determining the composition of cocoa powder. So far,

such measurements have not yet been reported in the literature, although instrument manufacturers report that cocoa powder can be analyzed with their NIR instruments, and also in the above-mentioned manuscript (personally handed over to us) results are reported on fat and moisture content determination in cocoa powder.

The present study aims at finding the possibility for applying the NIR technique for the rapid determination of fat, protein, and carbohydrate content in cocoa powder; and, at the same time, to predict accuracy for the different components, to choose the most suitable form of the regression equations, determine the parameters (characteristic wavelengths, coefficients, and constants) and last, but not least, to examine the achievable accuracy increase by studies on the transformed reflectance spectra.

The results of these studies will hopefully enable us to develop a single-purpose instrument or to extend the usage of another one to be able to perform such measurements.

## 1. Materials and methods

Since the NIR technique is strictly correlative, the accuracy of the analytical data of the calibration samples are of critical importance. In order to determine the relationship between optical properties and composition we needed a set of calibration samples in which fat, protein, and carbohydrate content values varied between minimum and maximum values—possibly equally distributed—of samples found in every day practice. Consequently these cocoa powder samples for calibration were carefully prepared at the Centre of Food Control and Analysis of the Ministry of Agriculture and Food, Budapest.

### 1.1. *Preparation of the cocoa powder samples*

The cocoa powder samples needed for the elaboration of the NIR technique for a rapid, instrumental composition analysis were made up from four ingredients, namely:

original cocoa powder,
skim milk powder,
starch,
cocoa butter.

The original cocoa powder used corresponded to the requirements in the Hungarian Standard (1975b). For skim milk powder the requirements were described in the Hungarian Standard (1976). The starch used fulfilled the requirements in the Hungarian Standard (1963). The original cocoa powder

and cocoa butter were specially made for our purposes in the Chocolate Factory, Szerencs.

The samples were specially prepared by mixing original cocoa powder, cocoa butter, skim milk powder and starch in predetermined ratios in order to obtain calibration samples to fulfil the requirements mentioned above. The ingredients of powder form were blended and cocoa butter was added in petroleum ether solution, the solvent was evaporated and then the sample was carefully homogenized in a mortar.

The carefully analyzed ingredients were mixed in predetermined quantities by weighing so that the composition of the cocoa powder samples could be calculated. The composition of the different samples was re-analyzed to make sure that the calculated data corresponded to the analytical data.

The fat, protein, and carbohydrate content were determined on the basis of the corresponding Hungarian Standard (1975a).

For calibration 20 samples were made in the following way:

- samples Nos. 1–5: fat content was increased by adding 2, 4, 6 and 8 mass % cocoa butter to cocoa powder to make it up to 100 mass %;
- samples Nos. 6–10: protein content was increased by adding 1, 2, 3 and 4 mass % skim milk powder to cocoa powder to make it up to 100 mass % (we regret to note that simultaneously the starch content was also increased as a result of starch content of milk powder);
- samples Nos. 11–15: carbohydrate content was increased by adding 2, 4, 6 and 8 mass % starch to cocoa powder to make it up to 100 mass %;
- samples Nos. 16–20: ingredients were simultaneously increased in four steps by adding 2 mass % cocoa butter, 1 mass % skim milk powder, and also 2 mass % starch to cocoa powder per step to make it up in each step to 100 mass %.

We placed 10 $cm^3$ of cocoa powder sample into the sample holder (cell) of 38 mm diameter with a 1.2 mm thick special quartz window (type G. E. 124) on one side and pressed with a pressure of about 10 mPa from the other. For illumination a beam (the cross-section was not quite circular) was used, the diameter of which varied between 15 to 20 mm at an incident angle of 0° through the quartz window (the beam was perpendicular to the surface of the sample). Four lead sulfide detectors (sensors) were equally spaced around the incident beam to measure the radiation diffusely reflected by the sample at 45°.

Each of the 20 different cocoa powder samples prepared at the Centre of Food Control and Analysis of the Ministry of Agriculture and Food, Budapest, were packed into three cells, thus we obtained a total of 60 samples to analyze and to use for calibration. Having three replicas from the 20 original samples, we had the opportunity to reduce the errors by averaging the measurements.

### 1.2. *The NIR spectrophotometer used*

The near infrared reflectance properties of cocoa powder samples were measured with a "one of a kind" computerized spectrophotometer built at USDA-BARC-West Instrumentation Research Laboratory, Beltsville. This instrument is built around a *Cary* Model 14 prism-grating monochromator with optics optimized for the near infrared. The monochromator is coupled to a digital computer for collecting and analysis of data. The instrument is operated in a single-beam mode with a reference spectrum stored in the computer.

The monochromator was operated with slits at 2 mm giving an effective bandpass of 7 nm. The region scanned was 1 000 to 2 638.4 nm. The signal from the lead sulfide detectors were amplified, digitized, and fed to the digital computer. Reflectance data were collected every 0.2 nm with 256 digital conversions per point. Thus, we got 8 192 reflectance points for the whole wavelength region. The scanning speed was 10 nm per s, so the time period between two data collections was 20 ms; with one half of the time for 256 readings during the "light period", and the other half to measure the "dark period" signal from the AC coupled amplifier in order to restore the absolute amplitude. To take a complete reflectance spectrum of a sample required about 165 s.

Ceramic material was used as a reference standard because its reflectance is the same for all wavelengths from 1 000 nm to 2 700 nm. The reflected signal from the ceramic standard was stored in the computer and used to divide the reflected signal from the cocoa powder samples. This gives a resultant curve of true diffuse directional-conial reflectance relative to the ceramic standard. (The term directional-conical reflectance is meant as a ratio of reflected flux collected through a conical solid angle to essentially collimated incident flux.) The computer corrects for the reflectance of the ceramic standard to give the real value of the diffuse directional-conical reflectance factor (R); the ratio of the flux actually reflected by a sample surface to that which would be reflected into the same reflected-beam geometry by an ideal (lossless), perfectly diffuse (lambertian) standard surface irradiated in exactly the same way as the sample. The diffuse directional-conical reflectance factor data were recorded at each measured reflectance point for each of the cocoa powder samples, and the spectral data were stored on magnetic tapes for further processing. A new reference signal from the ceramic standard was stored in the computer about once each hour to minimize errors from long-term drift.

For data processing, the 8 192-point spectral curves were smoothed by a running average of 21 points at each point and shrunk to 1 024-point curves by choosing every eighth point and averaging it with adjacent points on either side. Compressed spectral curves were transformed to log (1/R) curves and recorded along with the compositional data for each sample. The transformation of the R spectral curves to log (1/R) proved useful because the log (1/R)

function gives a linear correlation with the concentration of a given measured component. Plotting log (1/R) as a function of wavelength gives a spectral curve that is comparable to an absorption curve having peak readings at wavelengths that correspond to absorption bands in the sample. Preliminary studies indicated that performance could be improved by using the second derivative of the log (1/R) spectral curve rather than log (1/R). Therefore, all data were analyzed by use of the second derivative transformation of each wavelength point. The second derivative calculation was incorporated into the computer program for the linear regression analysis. A stepwise multiple-regression program was used also to analyze the data and determine the optimum wavelengths and other important parameters for predicting fat, protein, and carbohydrate content in cocoa powder samples.

Because changes in reflectance are small, even from relatively large changes in composition, reflectance data must be recorded with high precision. For this reason, the resolution we used at the measurements was 0.0000305 log (1/R) unit [1 log (1/R) corresponds to 32 768 steps].

The definition of the standard error of calibration used in the discussion is:

$$\sqrt{\frac{\sum_{i=1}^{n}(Q_{si}-Q_{ci})^2}{n-1-p}}$$

where

$Q_s$ is the respective quality parameter (dependent variable) determined by traditional standard methods,

$Q_c$ is the same quality parameter computed from the regression equation,

$n$ is the number of samples,

$p$ is the number of independent variables.

The standard error of calibration is derived by comparing the laboratory values of one set of samples to the instrument values of that same set when creating the calibration equation. The standard error of prediction is obtained by comparing the laboratory values of a second set of samples to the instrument percent reading of that second set and verifying the existing calibration.

All measurements described were carried out in an air-conditioned laboratory at 296 K (+23 °C).

## 2. Results

The compositional data, namely fat, protein, and carbohydrate content, of the 20 cocoa butter samples are summarized in Table 1.

We selected the log (1/R) spectra of three cocoa powder samples with high fat, with high protein, and with high carbohydrate content, respectively,

Table 1

*The compositional data of the 20 cocoa powder samples*

| Sample No. | Protein content (mass %) | Fat content (mass %) | Carbohydrate content (mass %) |
|---|---|---|---|
| 1 | 20.00 | 10.00 | 38.00 |
| 2 | 19.60 | 11.80 | 37.24 |
| 3 | 19.20 | 13.60 | 36.48 |
| 4 | 18.80 | 15.40 | 35.72 |
| 5 | 18.40 | 17.20 | 34.96 |
| 6 | 20.00 | 10.00 | 38.00 |
| 7 | 20.459 | 9.73 | 38.324 |
| 8 | 20.918 | 9.46 | 38.648 |
| 9 | 21.377 | 9.19 | 38.972 |
| 10 | 21.836 | 8.92 | 39.296 |
| 11 | 20.00 | 10.00 | 38.00 |
| 12 | 19.60 | 9.80 | 39.24 |
| 13 | 19.20 | 9.60 | 40.48 |
| 14 | 18.80 | 9.40 | 41.72 |
| 15 | 18.40 | 9.20 | 42.96 |
| 16 | 20.00 | 10.00 | 38.00 |
| 17 | 19.566 | 10.606 | 38.990 |
| 18 | 19.318 | 12.66 | 39.608 |
| 19 | 18.977 | 13.99 | 40.412 |
| 20 | 18.636 | 15.32 | 41.216 |

which are shown in Figs. 1–3. The second derivative curves of the corresponding log (1/R) spectra are also plotted in the same figures.

Figures 4–6 show the log (1/R) spectra of the three ingredients, such as cocoa butter, skim milk powder, and starch used to enrich the original cocoa powder in fat, protein, and carbohydrate, as well as the second derivatives of the corresponding log (1/R) spectra.

The figures illustrate that the second derivatives of the log (1/R) spectra do show a much greater difference than log (1/R) between samples of different composition, sharpening the details in the curves.

The relationship between log (1/R) values and fat, protein, and carbohydrate content of the 20 cocoa powder samples (60 spectra, three replicas of each sample) was determined by use of an iterative procedure with the multiple regression analyses. The computer program determined the characteristic wavelengths and the coefficients that gave the best fit to the following

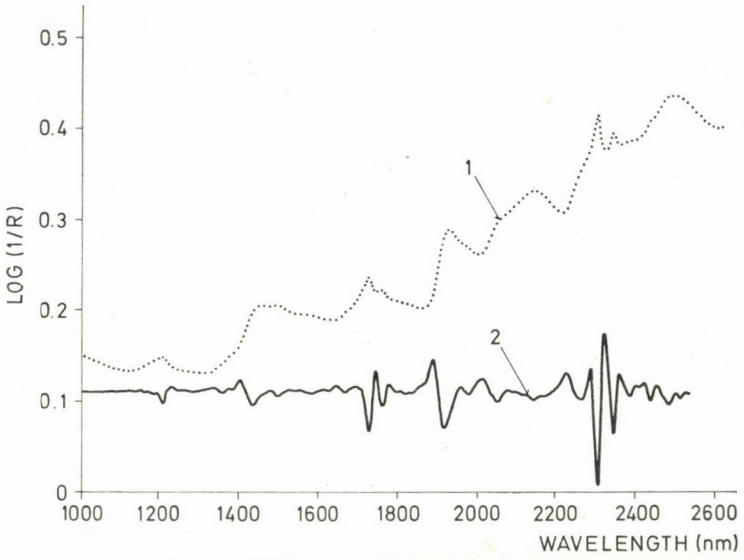


Fig. 1. The log (1/R) spectrum and the second derivative of cocoa powder sample No. 5 containing high percentage of oil. The measuring geometry: 0°/45°. Diameter of the illumination beam: 20/15 mm. Spectral bandpass: 7 nm. Spectra were taken in 0.2 nm steps, compressed, then recorded in 1.6 nm steps. Vertical resolution 0.00003 log (1/R) unit. 1: log (1/R) spectrum of sample No. 5; 2: Second derivative of the log (1/R) spectrum

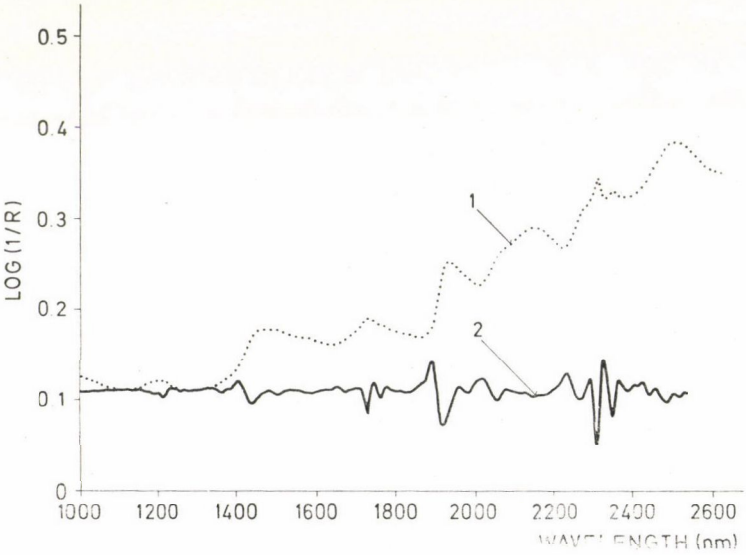


Fig. 2. The log (1/R) spectrum and the second derivative of cocoa powder sample No. 10 containing high percentage of protein. The measuring geometry: 0°/45°. Diameter of the illumination beam: 20/15 mm. Spectral bandpass: 7 nm. Spectra were taken in 0.2 nm steps, compressed, then recorded in 1.6 nm steps. Vertical resolution 0.00003 log (1/R) unit. 1: log (1/R) spectrum of sample No. 10; 2: Second derivative of the log (1/R) spectrum

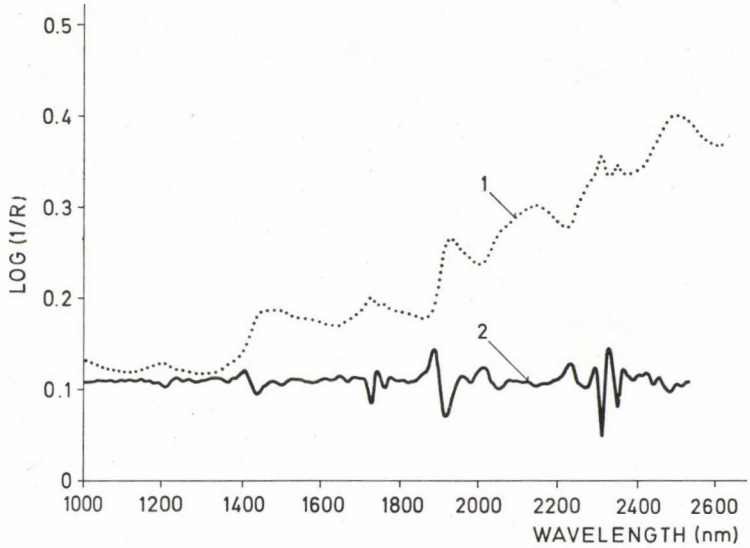


Fig. 3. The log (1/R) spectrum and the second derivative of cocoa powder sample No. 15 containing high percentage of carbohydrate. The measuring geometry: 0°/45°. Diameter of the illumination beam: 20/15 mm. Spectral bandpass: 7 nm. Spectra were taken in 0.2 nm steps, compressed, then recorded in 1.6 nm steps. Vertical resolution 0.00003 log (1/R) unit. 1: log (1/R) spectrum of sample No. 15; 2: Second derivative of the log (1/R) spectrum

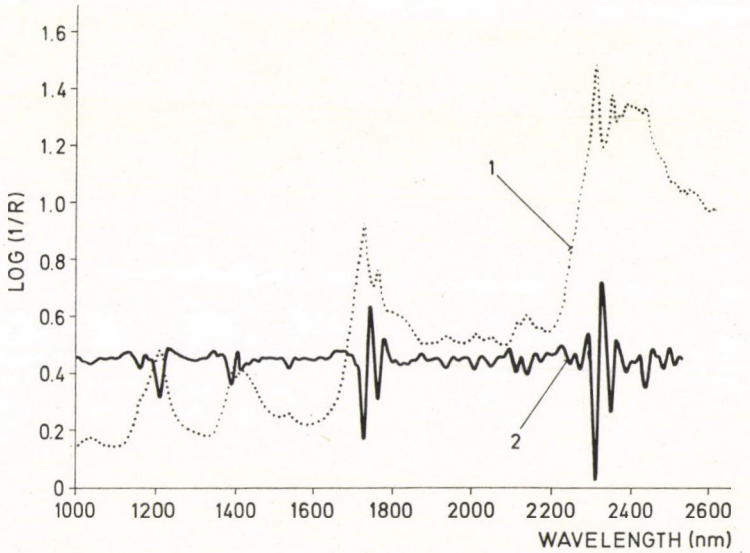


Fig. 4. The log (1/R) spectrum and the second derivative of cocoa butter. The measuring geometry: 0°/45°. Diameter of the illumination beam: 20/15 mm. Spectral bandpass: 7 nm. Spectra were taken in 0.2 nm steps, compressed, then recorded in 1.6 nm steps. Vertical resolution 0.00003 log (1/R) unit. 1: log (1/R) spectrum of cocoa butter; 2: Second derivative of the log (1/R) spectrum

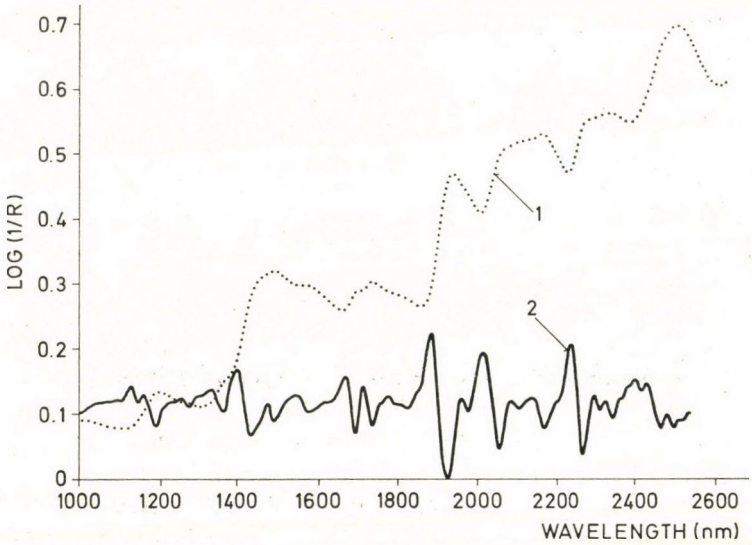


Fig. 5. The log (1/R) spectrum and the second derivative of skim milk powder. The measuring geometry: 0°/45°. Diameter of the illumination beam: 20/15 mm. Spectral bandpass: 7 nm. Spectra were taken in 0.2 nm steps, compressed, then recorded in 1.6 nm steps. Vertical resolution 0.00003 log (1/R) unit. 1: log (1/R) spectrum of skim milk powder; 2: Second derivative of the log (1/R) spectrum

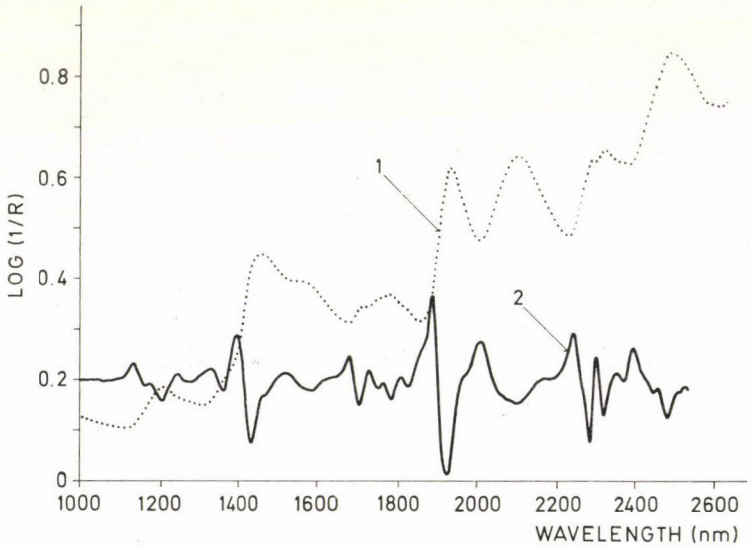


Fig. 6. The log (1/R) spectrum and the second derivative of starch. The measuring geometry: 0°/45°. Diameter of the illumination beam: 20/15 mm. Spectral bandpass: 7 nm. Spectra were taken in 0.2 nm steps, compressed, then recorded in 1.6 nm steps. Vertical resolution 0.00003 log (1/R) unit. 1: log (1/R) spectrum of starch; 2: Second derivative of the log (1/R) spectrum

equation for each measured component:

$$Q_{1i} = K_{0i} + K_{1i} V_{\lambda 1i} + K_{2i} V_{\lambda 2i}$$

where

$Q_{1i}$ stands for the composition parameters (fat, protein, and carbohydrate);

$K_{0i}$, $K_{1i}$ and $K_{2i}$ are coefficients and constants, resp.,

$\lambda_{1i}$ and $\lambda_{2i}$ are characteristic wavelengths;

$V_{\lambda 1i}$ and $V_{\lambda 2i}$ are the log (1/R) values belonging to these characteristic wavelengths.

We used only two term equations because of the limited quantity of calibration samples in order to avoid overfitting.

The results are summarized for fat, protein and carbohydrate content in Table 2 containing the characteristic wavelengths, the coefficients and constants of regression equations as well as standard errors of calibration and multiple correlation coefficients.

We tried to find a relationship between composition parameters and log (1/R) values at other absorption bands of the above-mentioned components using the same equation form. The results in standard error of calibration and multiple correlation coefficient were almost as good as those shown in Table 2.

Table 2

*Summary of linear regression analyses relating data from chemical analyses and log (1/R) values measured at two characteristic wavelengths for 20 cocoa powder samples, searching 1 000 to 2 500 nm*

Equation form: $Q_{1i} = K_{0i} + K_{1i} V_{\lambda 1i} + K_{2i} V_{\lambda 2i}$

| | For fat content | For protein content | For carbohydrate content |
|---|---|---|---|
| Characteristic wavelength $\lambda_1$ (nm) | 1 739.2 | 2 059.2 | 2 124.8 |
| Characteristic wavelength $\lambda_2$ (nm) | 1 724.8 | 2 107.2 | 2 244.8 |
| Constant $K_0$ | 0.45559 | 47.511 | 39.113 |
| Coefficient $K_1$ | −1 734.2 | 2 305.4 | 1 367.6 |
| Coefficient $K_2$ | 1 748.0 | −2 256.4 | −1 394.1 |
| Standard error of calibration (mass %) | 0.199 | 0.340 | 0.406 |
| Multiple correlation coefficient | 0.9972 | 0.9409 | 0.9807 |

Following this, we endeavored to increase accuracy by transforming the data to second derivative of the log (1/R) spectra. The relationship between transformed spectral data and composition parameters were studied in the

following form of equation:

$$Q_{2i} = K_{3i} + K_{4i} \frac{V''_{\lambda 3i}}{V''_{\lambda 4i}}$$

where

$Q_{2i}$ stands for the composition parameters;

$K_{3i}$ and $K_{4i}$ are coefficients and constants, resp.,

$V''_{\lambda 3i}$ and $V''_{\lambda 4i}$ are the values of the second derivatives of the log (1/R) spectra at $\lambda_{3i}$ and $\lambda_{4i}$ characteristic wavelengths.

Characteristic wavelengths, constants, and coefficients of these single-term regression equations, as well as standard errors of calibration and correlation coefficients, are summarized in Table 3.

Table 3

*Summary of linear regression analyses relating data from chemical analyses and values of the second derivative of log (1/R) curves at two characteristic wavelengths for 20 cocoa powder samples, searching 1 000 to 2 500 nm*

Equation form: $Q_{2i} = K_{3i} + K_{4i}(V''_{\lambda 3i}/V''_{\lambda 4i})$

| | For fat content | For protein content | For carbohydrate content |
|---|---|---|---|
| Characteristic wavelength $\lambda_3$ (nm) | 1 763.2 | 2 054.4 | 2 088.0 |
| Characteristic wavelength $\lambda_4$ (nm) | 1 633.6 | 2 286.4 | 1 763.2 |
| Constant $K_3$ | −2.9041 | −3.7769 | 29.209 |
| Coefficient $K_4$ | −21.257 | 74.525 | 11.236 |
| Segment (wvl. range for averaging) (nm) | 6.4 | 6.4 | 6.4 |
| Gap for numerator (nm) | 14.4 | 25.6 | 44.8 |
| Gap for denominator (nm) | 33.6 | 46.4 | 44.8 |
| Standard error of calibration (mass %) | 0.134 | 0.186 | 0.233 |
| Correlation coefficient | 0.9986 | 0.9817 | 0.9938 |

In this table, the gaps for numerator and denominator wavelengths, as well as segments, are listed. The gaps are the distances among the three wavelengths used for producing the second derivative, while segment is the wavelength region in which the measured spectral data are averaged.

The fat, protein, and carbohydrate have absorption bands in several regions of the near infrared, so we tested selected regions with similar results.

The results for fat, protein and carbohydrate determination using log (1/R) values and the second derivative ratio, shown in Tables 2 and 3, are graphed in Figs. 7–12.

The reproducibility was determined by comparing the spectra from the three replicas of the original twenty samples. Reproducibility was charac-

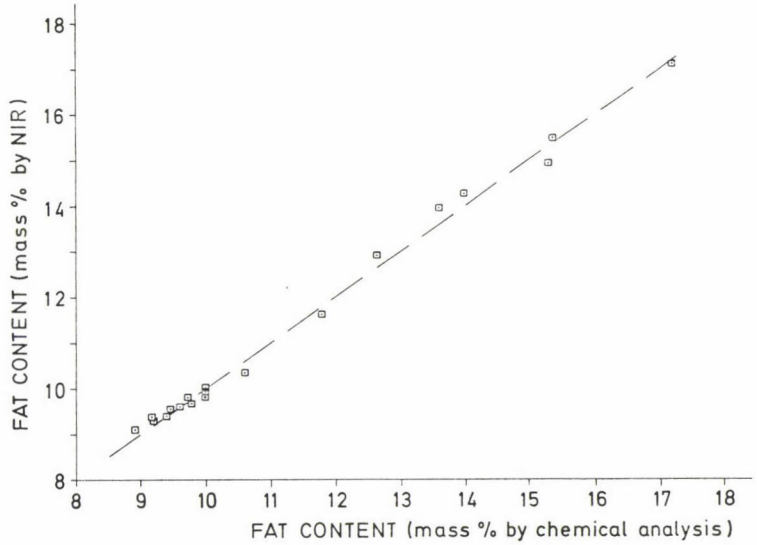


Fig. 7. Relationship between fat content determined by chemical analysis and predicted value from multiple regression log (1/R) data at two characteristic wavelengths

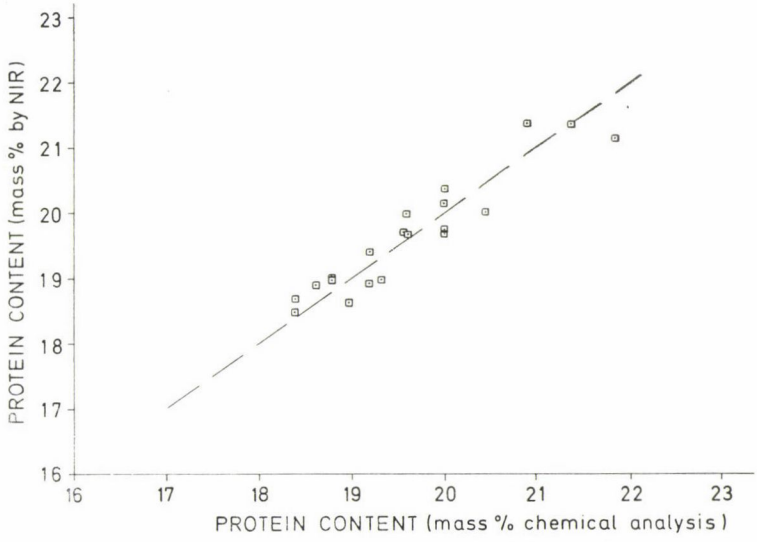


Fig. 8. Relationship between protein content determined by chemical analysis and predicted value from multiple regression log (1/R) data at two characteristic wavelengths

terized by the average of the standard deviations of the predicted data of the values which can be seen for the different components and different equation forms in Table 4.

Table 4

*Reproducibility and repeatability for cocoa powder samples expressed as the average of the standard deviations of the predicted data for fat, protein and carbohydrate content using multiterm log (1/R) (1) and second derivative (2) forms of equations*

| | Reproducibility | Repeatability |
|---|---|---|
| $Q_{1\text{FAT}}$ | 0.156 | 0.067 |
| $Q_{2\text{FAT}}$ | 0.187 | 0.099 |
| $Q_{1\text{PROTEIN}}$ | 0.519 | 0.117 |
| $Q_{2\text{PROTEIN}}$ | 0.230 | 0.169 |
| $Q_{1\text{CARBOHYDRATE}}$ | 0.513 | 0.099 |
| $Q_{2\text{CARBOHYDRATE}}$ | 0.484 | 0.233 |

The repeatability was determined by recording ten spectral scans on each of three selected samples without changing the position of the samples. The selected samples were those with high fat, protein, and carbohydrate content, respectively, (Sample No. 5, No. 10 and No. 15). The repeatability was characterized by the average of the standard deviations of the predicted data of ten scans for the three samples. The repeatability data for the different components and different equation forms are shown in Table 4.

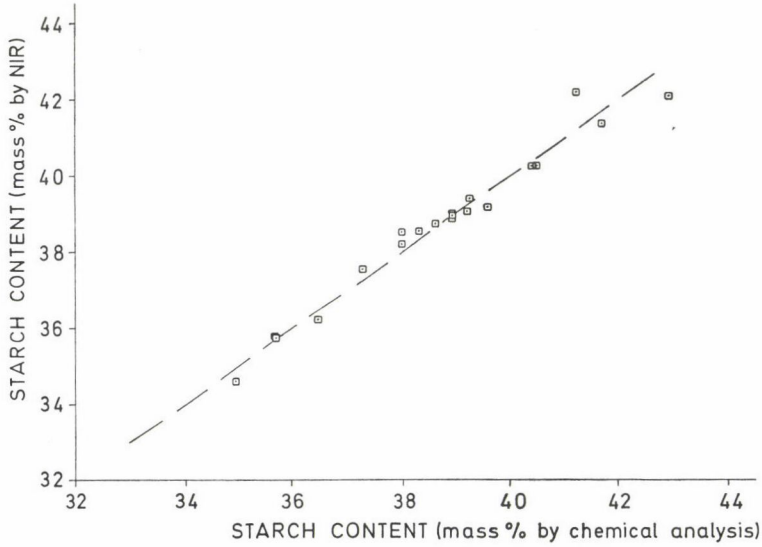


Fig. 9. Relationship between carbohydrate content determined by chemical analysis and predicted value from multiple regression log (1/R) data at two characteristic wavelengths

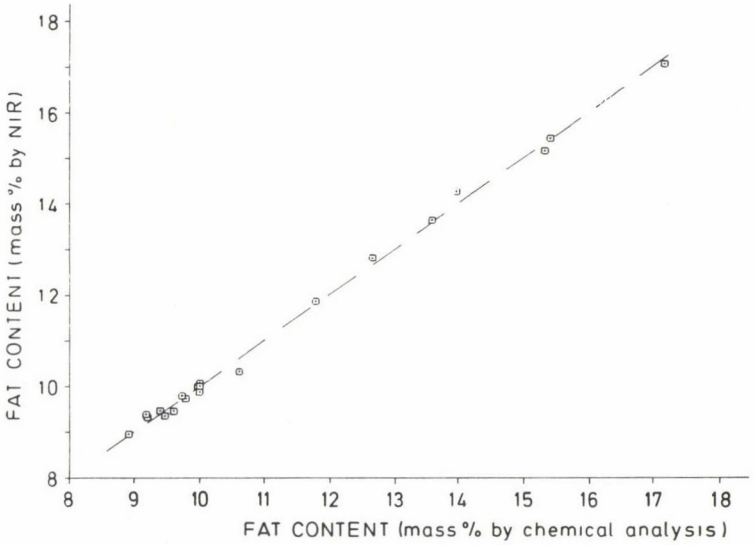


Fig. 10. Relationship between fat content determined by chemical analysis and predicted value from linear regression of the ratio of the second derivatives of the log (1/R) curves at two characteristic wavelengths

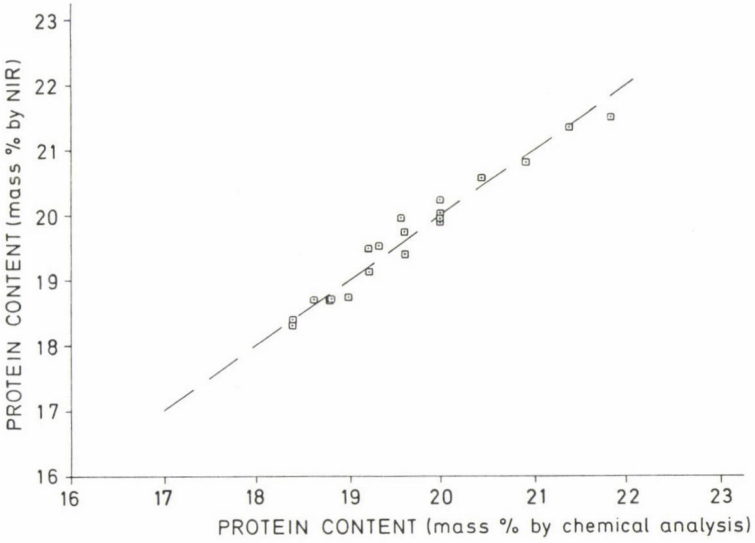


Fig. 11. Relationship between protein content determined by chemical analysis and predicted value from linear regression of the ratio of the second derivatives of the log (1/R) curves at two characteristic wavelengths

We would like to note that the data in Table 4 characterize one single scan on cocoa powder, while the data in Table 2 and Table 3 characterize the average of the scans on three replicas of the same original samples.

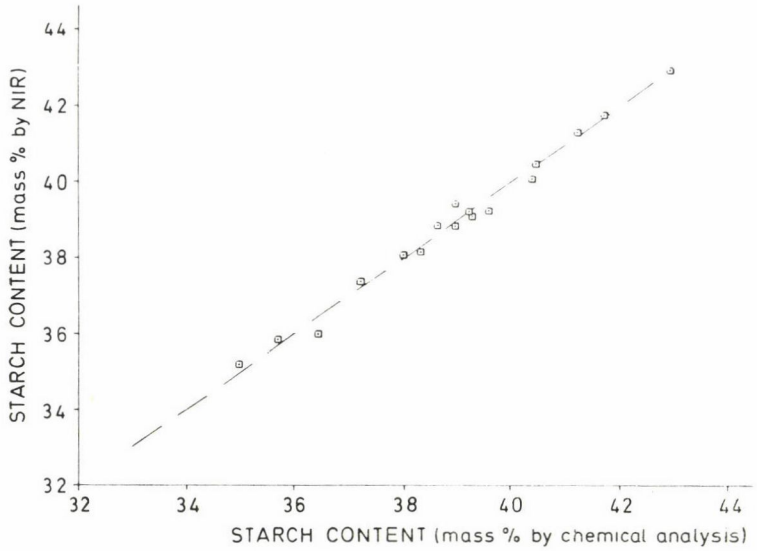


Fig. 12. Relationship between carbohydrate content determined by chemical analysis and predicted value from linear regression of the ratio of the second derivatives of the log (1/R) curves at two characteristic wavelengths

## 3. Conclusions

As can be seen in Table 1, the fat content in our cocoa powder samples has a range from 8.92 to 17.20 mass %, the change is about 92% in fat content.

The range of protein content is from 18.40 to 21.84 mass %; the change is about 19% in protein content. For carbohydrate, the range is from 34.96 to 42.96; the change is about 23% in carbohydrate content.

The particle size distribution of the samples was not tested. In accordance with the experiences gained by MINER and co-workers (1980) we found that particle size was very small and uniform, thus no disturbance was noted.

In our studies, no samples were omitted for the calculations, although the results could have been improved by omitting samples with a large deviation.

Our experiments showed satisfactory accuracy for fat, protein and carbohydrate prediction in cocoa powder using two-term linear equations containing the log (1/R) values measured at two different characteristic wavelengths; however, higher accuracy was obtained with the transformation of log (1/R) spectra; namely, by using the second derivatives of the spectra. In this case, we actually used a single-term equation where the value of the second derivative of the log (1/R) spectra at the first characteristic wavelengths was divided

by the value of the second derivative of the log (1/R) spectra at the second characteristic wavelength.

Using other wavelengths in the $Q_{2i}$ equation form we could find some far better results in terms of standard error of calibration, but reproducibility and repeatability examinations gave extremely bad results clearly pointing out that we are faced with overfitting problems; this means that the equations using such "pseudo characteristic" wavelengths described relationship between composition and optical parameters only for this set of samples, and not the "real" relationship provided by nature. These "pseudo characteristic" wavelengths giving such "good" results were not the ones where the examined components had their absorption bands. Naturally they were omitted in our further studies.

MINER and co-workers (1980) in the afore-mentioned study dealt with moisture and fat content determination in cocoa powder, in our investigation, however, protein and carbohydrate content determination was also included.

The colour of cocoa powder is known to vary as a result of different factors such as variety, geographical location of production, soil conditions, climate, *etc.* It has been experienced with other foodstuffs tested that even though there were differences in the visible spectra there were no differences in the spectral range above 1000 nm. Assuming the same in the case of cocoa powder, samples of different colour were not included in our calibration samples.

The correlation results presented here indicate that infrared reflectance measurements can be related to the composition analysis of cocoa powder. These calibration results have not been tested against unknown samples, but the very high correlation coefficients indicate that it should be possible to predict fat, protein, and carbohydrate content with high accuracy using near infrared reflectance techniques.

These results were obtained by exploring only two of many possible data treatments and using only the optical geometry and sample size dictated by the spectrophotometer. The reproducibility and repeatability values indicate that performance could be significantly improved by recording more replicas, by increasing the diameter and intensity of the illuminating beam, and it is also possible that the performance could be improved with other data treatments.

*

Thanks are due to Ms. Joyce SHAFFER of the USDA-BARC-West Instrumentation Research Laboratory, Beltsville for her conscientious technical assistance in performing measurements and data processing.

We are most grateful to the Institute of State Food Control and Analysis, Miskolc for providing us with specially prepared cocoa powder and cocoa butter samples and the useful advice given.

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Addresses of the authors:

Dr. Károly J. KAFFKA — Central Food Research Institute, H-1022 Budapest, Herman Ottó út 15., Hungary

Karl H. NORRIS — USDA-BARC-West, Instrumentation Research Laboratory, Beltsville, Maryland 20705, USA

Dr. Ferenc KULCSÁR, Ms. Imelda DRASKOVITS — Centre of Food Control and Analysis of the Ministry of Agriculture and Food, H-1051 Budapest, Guszev utca 25., Hungary

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# YEAST IN ALCOHOL PRODUCTION AND BREAD MAKING*

H. SUOMALAINEN

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*Fuel alcohol.* The energy crisis has stimulated an unprecedented development in the research into how oil products can be replaced by renewable natural resources. Interest has been directed towards the enzymatic hydrolysis and fermentation to ethanol of cellulose materials. However, really extensive industrial applications are unlike to materialise in the near future. The wood conversion industries in Northern Europe are already major consumers of wood, and experts in Finland believe that the exploitation of wood has almost reached its limit.

*Beverages.* Much of the work studying the properties of alcoholic beverages has been concerned with the factors responsible for the formation of the aroma, and many investigations have confirmed that a great variety of metabolic products is formed in yeast cells during fermentation. With regard to the esters, our results show that part of the compounds formed by the yeast remains in the cell and part permeates through the cell membrane into the medium. Consequently, the ester composition may be different in the distillate if the distillation of the medium is carried out in the presence of the yeast or after its removal.

*Baking.* The latest estimate for the annual production of baker's yeast exceeds 1 400 000 tons (fresh weight) and is growing only slowly if at all in the industrialized countries. The developing countries, *e.g.* in Africa and the Islamic regions, have started to show interest in their own yeast production. In Finland consumption of baker's yeast has reached a steady level at nearly 2 kg per person per year, with a third being used in home baking. The consumption of much rye bread is a Finnish national characteristic.

## Fuel alcohol

The energy crisis has made the terms exploitation of renewable natural resources, liquefaction of biomass, fuel alcohol and gasohol known to the public at large, and in many parts of the world unprecedented efforts are being concentrated on the problems associated with the liquefaction of biomass. One link in the long and complicated process is familiar: the alcoholic fermentation with yeast. The direction of the fermentation research is rapidly moving towards practical application, primarily to develop yeast strains that will work faster, at higher temperatures and which can tolerate higher concentrations of sugar and alcohol. This yeast research and the developments in the process itself are aimed at the next generation of production units since,

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despite the increasing cost of oil, fuel alcohol produced in conventional plants is experiencing a hard price squeeze. The stages of the processes involved and the whole production philosophy need to be reconsidered from the beginning to the end. Three main principles must be kept in mind when modifying the processes:

- efficiency of conversion to ethanol (yield),
- energy consumption (conservation),
- effluent pollution (environmental protection).

However, plants continue to be built using conventional technology and ignoring these principles, Brazil being a prime example. The alcohol industry there is based mainly on sugar cane, but also on manioc. Waste bagasse from the cane, when burnt, provides so much energy that it is not thought necessary to include energy-saving designs in the process (the distillation columns are not even insulated). Waste water is disposed of simply by pumping it back to the cane plantations, and the alcohol yield is kept reasonably high by adding penicillin and pentachlorophenol to the fermantation. The possible consequences of recirculating these bacteriostats from the fermentation to the plantation and back, with the risk of continuous enrichment in the environment, have not been evaluated. As is well known, Brazil has invested great sums of money in alcohol production, and the government's National Alcohol Programme *Próalcool*, which was launched in 1975, involves a really vast scheme (STUMPF, 1978). It is intended that during 1980 20% of all gasoline used will be replaced by alcohol, and that in the following year one vehicle in six will be running on neat alcohol and the remainder on gasoline containing 20% alcohol. Brazil would seem to be a country with many of the advantages needed to achieve these aims. The country is big, has good supplies of water and a favourable climate. Calculations show that cultivating 2% of the land area with sugar cane would provide raw material for enough alcohol to replace all imported oil, which is currently 80% of the total consumption. Of course, on the European scale this 2% of Brazil's land area would be substantial, about 1/3 of the German Federal Republic. This gives some idea of the magnitude and the possibilities in Brazil.

The USA has its own plans for the production of gasoline containing a proportion of ethanol (ANONYM, 1980). The state of Nebraska, for example, has the problem of how to dispose of its excess maize production, which it intends to solve by producing fuel alcohol from it. This gasohol (10% ethanol + + 90% gasoline) scheme has attracted strong criticism, mainly regarding the energy balance of the process. Calculations have shown that using existing technology to produce ethanol requires 2.7 times as much energy as that contained in the product (HARALDSON, 1979). Its defenders, however, claim that each gallon of fuel alcohol used saves 0.7 gallon crude oil, but the public seems to have faith in the increased use of alcohol, and alcohol production is

attracting growing amounts of investment. However, if alcohol is to be produced and used as a motor fuel, enormous amounts of alcohol will be present around the world, and a heavy misuse can be expected (FORSANDER, 1980).

The use of ethanol as motor fuel is anything but a recent innovation. It goes back to the end of the last century (REESE, 1979). During the first decade of this century gasoline rapidly became cheap and plentiful. Nevertheless, the use of ethanol as motor fuel, either alone or mixed with gasoline has increased during times of crisis. During the Second World War in Finland military equipment and civilian transport were running partly on a gas made in the vehicle from wood and partly on bentyl, *i.e.* gasoline containing up to 25% ethanol. At that time the alcohol was produced from the waste liquor from sulphite mills. The production of sulphite alcohol increased some 20 fold during and after the war, with a maximum of 17 sulphite alcohol plants in operation. The first sulphite spirit factory built in Finland in 1920 is shown in Fig. 1. The sulphite spirit plants were the first ones to convert to the continuous fermentation process. The use of ethanol as an additive to motor fuel continued during the long years of austerity following the war. It was gradually reduced, however, and stopped in Finland completely after 1958. The production of sulphite spirit has also suffered a severe decline, largely because the sulphate process has increasingly replaced the sulphite process as a result of stricter environmental regulations. Consequently, the sulphite spirit distilleries have closed down one after another, so that at present only three are working in Finland (KAUKORANTA, 1981).

Countries where sugar cane, manioc, millet or maize cannot flourish are left with the possibility of using as carbohydrate source such cellulose materials

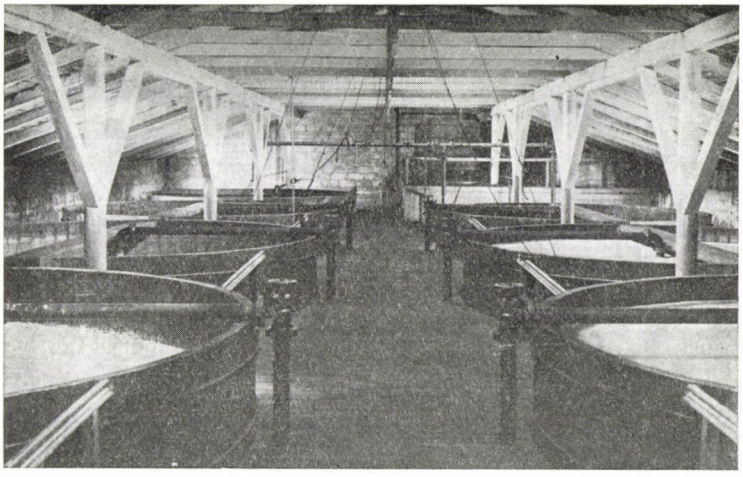

Fig. 1. Wooden fermentation vats in the first Finnish sulphite spirit plant built in 1920 at Tainionkoski in South-East Finland

as wood, straw and peat. The wood processing industries are substantial consumers of wood material, and according to some authoritative assessments in Finland, the amount of wood used is already now close to the replacement rate. Vast areas of Finland are forests. This is also true for Lapland (Fig. 2), although there a great deal of felling without replacement has occurred (Fig. 3). The timber from the recently scheduled "energy forests" will not become available for ten or twenty years, and even then it is estimated that it will satisfy only 10% of Finland's total energy needs (HUIKARI, 1979). The map

Fig. 2. Finland has vast forests also in North Finland (Photo: HAUTALA, H.)

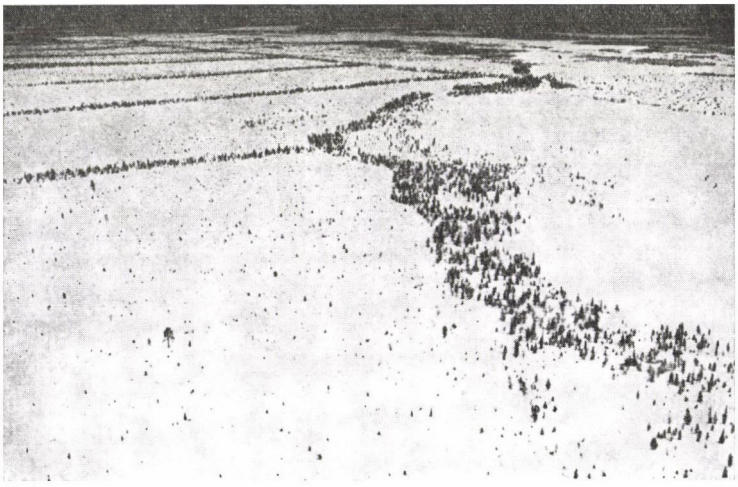

Fig. 3. It takes a long time to replace large areas of felled trees in Lapland (Photo: KEMILÄ, E.)

in Fig. 4 gives an idea of how far north Finland and the other Scandinavian countries are with reference to Canada and the USA (SUOMALAINEN, 1980). Figure 5 shows a section through a pine with a diameter of 33 cm only, which started its growth in the harsh conditions of Northern Finland many decades before Columbus discovered America. Moreover, it has recently been definitely demonstrated that the atmospheric pollution by sulphur compounds has begun to retard forest growth also in Finland (HUTTUNEN *et al.*, 1980).

When wood material is used as carbohydrate source, there are two obvious ways of hydrolysing it: acid or enzymatic hydrolysis. Acid hydrolysis is an old method that is known to be uneconomic and has been used in many countries only at times of crisis. Recent research, however, has blown new life into the method. A recent source of interest is the so-called extruder method, in which cellulose under high shear and at high temperatures is continuously hydrolysed by acid just before the cellulose pulp leaves a twin-screw extruder (ANONYM, 1979). This method requires only a short acid contact time, which reduces the likelihood of sugars formed being further broken down.

Enzymatic hydrolysis is being extensively researched in many countries (ENARI & MARKKANEN, 1977; LINKO, 1977). At present the main problem seems to be how to pre-treat the material rather than how to produce suitable cellulase enzymes. In order to act on the cellulose the enzyme must first reach it through the lignin envelope. This demands drastic and energy-consuming pre-treatment.

An additional problem is that both acid and enzymatic hydrolysis methods produce only dilute sugar solutions. This means that the distillation of the alcohol after fermentation will be expensive. Moreover, because the waste is

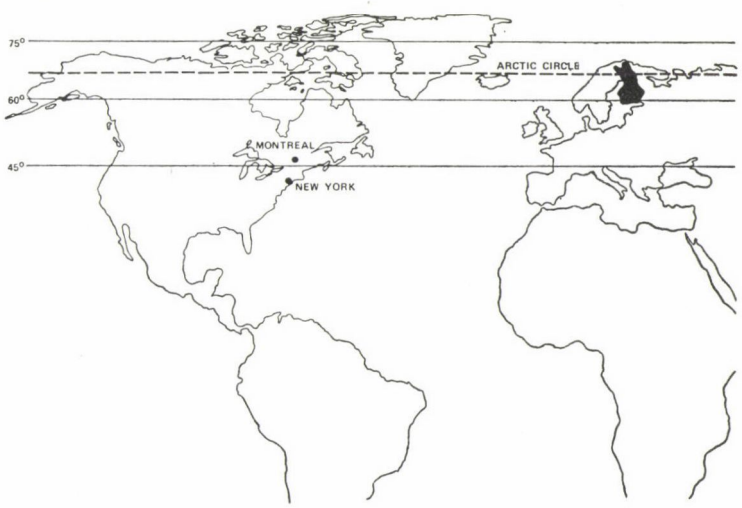


Fig. 4. The location of Finland and Scandinavia (SUOMALAINEN, 1980)

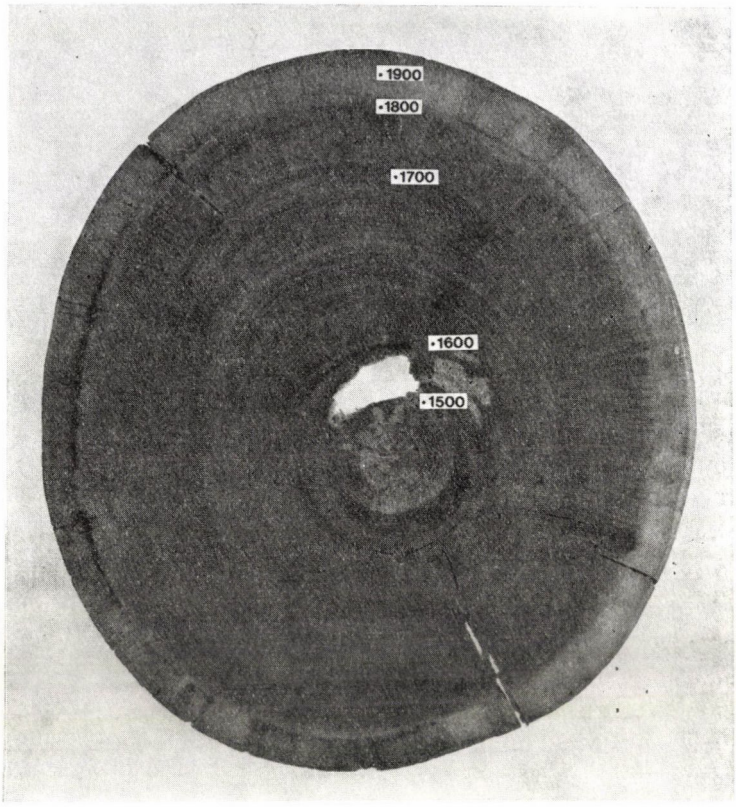


Fig. 5. 33 cm diameter section of a pine from Ivalo in North Finland (SUOMALAINEN, 1980)

so dilute, it will be uneconomic to evaporate the water and then use the residual lignin.

The future relative importance of enzymatic and acid hydrolysis in alcohol production can only be conjectured, but because of existing demands on timber resources it seems that the Northern countries will not be able to replace more than a limited proportion of gasoline with ethanol made from biomass. Indeed, in these countries more attention is likely to be directed towards the production and use of methanol, which can be made from many starting materials, including waste, by thermal and hydration reactions.

## Beverages

As is generally known, the organoleptic effect experienced when an alcoholic beverage is consumed, *i.e.* its aroma, is the result of the combined action of several hundred chemical compounds (NYKÄNEN & SUOMALAINEN,

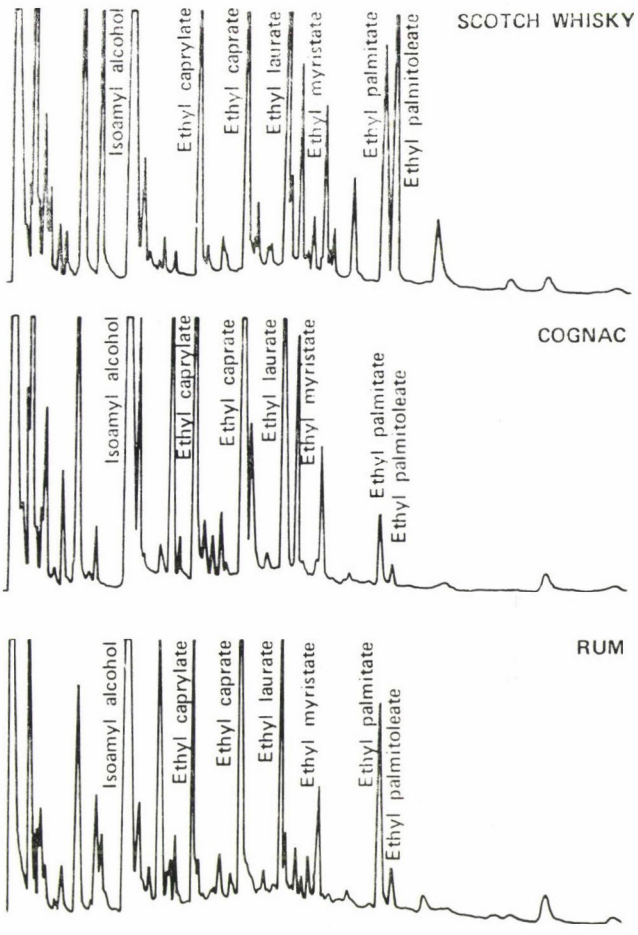


Fig. 6. Gas chromatograms of the major aroma compounds isolated from Scotch whisky, cognac and rum (SUOMALAINEN & LEHTONEN, 1979)

1981). Although it is quite easy to distinguish between the various drinks sensorily, it is extremely difficult even with the most up-to-date analytical methods to characterise the differences in their chemical composition. It is apparent from the chromatograms of the aroma compounds in Scotch whisky, cognac and rum (Fig. 6) that there is a marked qualitative similarity between the beverages.

A major goal of aroma research has been to discover the origin of the aroma components, and it is well established that (besides distillation and ageing in wooden casks) fermentation is in this respect the most important stage in beverage production. It is then that the main part of the aroma compounds in the finished product is formed. Being a single-cell organism yeast makes good contact with its surroundings (JUST, 1940), so that the

compounds utilized by the yeast can easily pass into the cell and the metabolites into the medium.

Yeast has been shown experimentally to have a central position in production of aroma compounds (SUOMALAINEN & LEHTONEN, 1979). It produces much the same compounds in nitrogen-free sugar fermentation as appear in alcoholic beverages (Fig. 7), although the amounts vary substantially (SUOMALAINEN & NYKÄNEN, 1966a). Isoamyl alcohol and phenetyl alcohol are quantitatively the largest aroma compounds in the sugar fermentation.

Figure 8 presents chromatograms of the aroma compounds isolated from genuine Spanish sherry and a wine of sherry type made from Finnish currants. Both were produced by the *Solera* method using *Jerez* yeast (SUOMALAINEN & NYKÄNEN, 1966b). It would be natural to assume that products from such

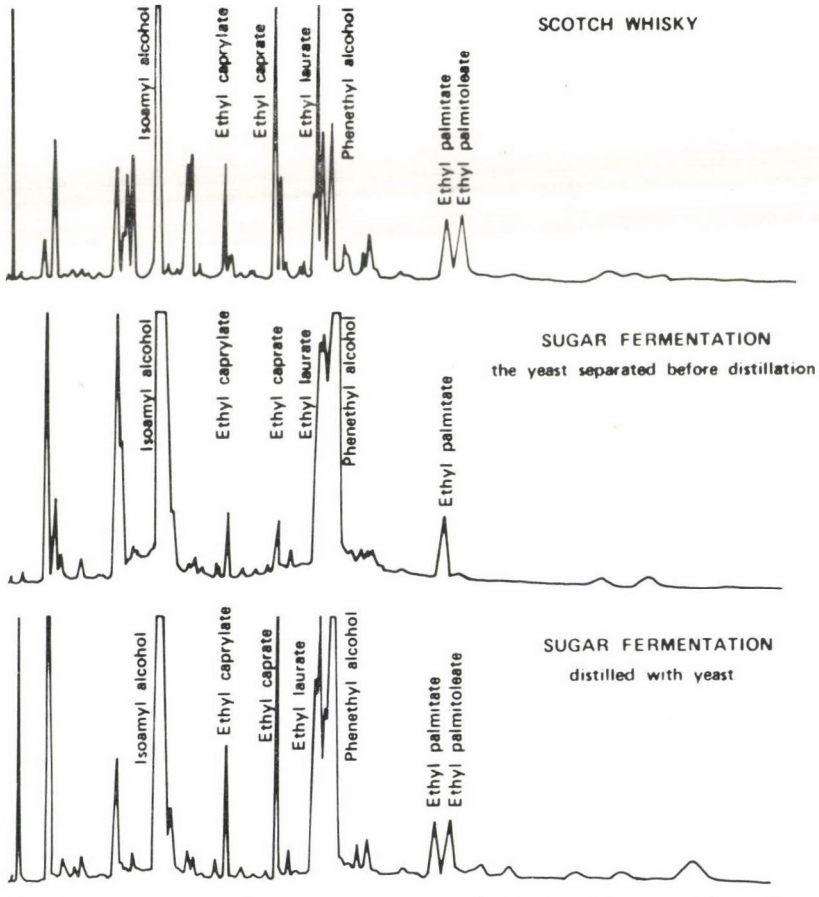


Fig. 7. Gas chromatograms of the aroma compounds produced by yeast in a nitrogen-free sugar fermentation and those present in a Scotch whisky (SUOMALAINEN & NYKÄNEN, 1966a)

different raw materials would have quite different chemical compositions, but such is not the case; on the contrary, they each contain surprisingly similar aroma compounds.

Neglecting ethanol, fuel alcohols compose the quantitatively largest group of compounds produced by yeast during fermentation. Depending on the kind of beverage the proportion of the main component, isoamyl alcohol, in this fraction is 40—70% (SIHTO *et al.*, 1962). Other major components include *n*-propyl alcohol, isobutyl alcohol and optically active amyl alcohol. The proportions formed depend on the yeast strain used.

A number of aldehydes are formed as intermediates during fermentation, which, because of their low sensory thresholds, make a considerable contribution to the aroma of the product. Fairly large amounts of aldehydes appear in fermentation mixtures, but the amounts decrease during distillation (PYKE, 1965).

Other important compounds include diketones, primarily diacetyl and 2,3-pentanedione. Diacetyl is formed from α-acetolactic acid by spontaneous decarboxylation (Fig. 9), and 2,3-pentanedione apparently analogously from α-aceto-α-hydroxybutyric acid (SUOMALAINEN & RONKAINEN, 1968). The thresholds of diketones are quite low, so that even small concentrations can

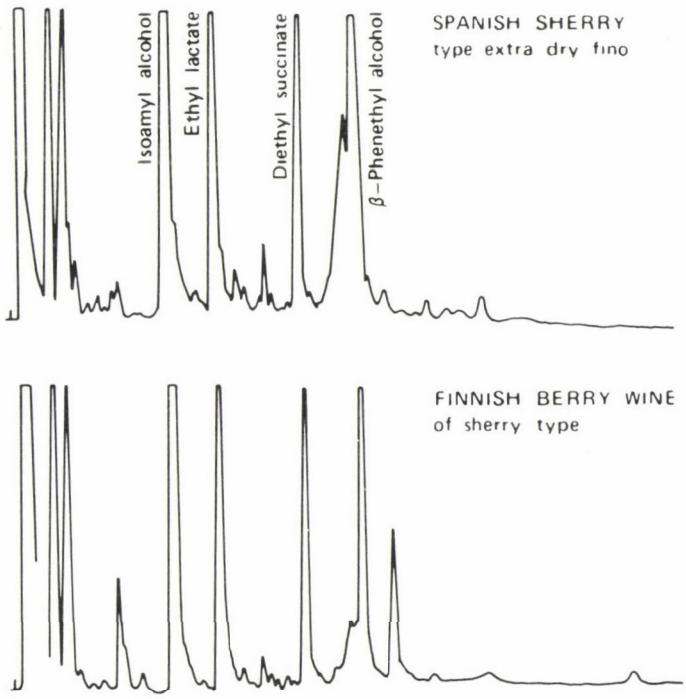


Fig. 8. Gas chromatograms of the aroma compounds of a Spanish sherry and a Finnish berry wine of sherry type (SUOMALAINEN & NYKÄNEN, 1966b)

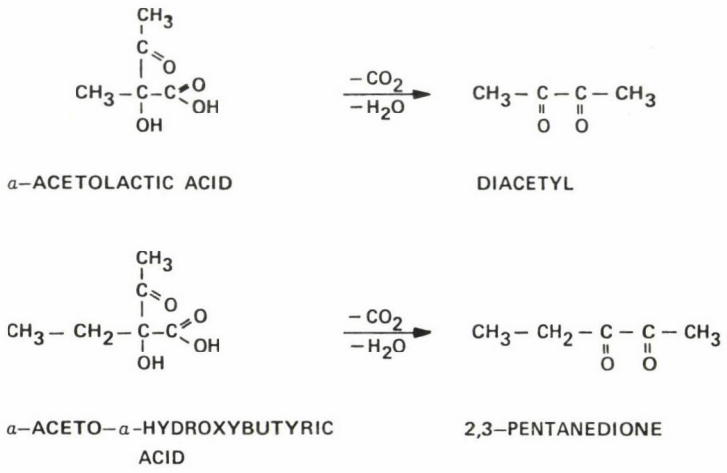


Fig. 9. Scheme for the formation of diketones in anaerobic fermentation

be perceived in the beverages. It is well known that elevated levels of diketones in beer easily lead to off-flavours.

As has been mentioned in connection with diketones, all aroma compounds do not necessarily have a pleasing effect in beverages. Investigations into the causes of off-flavours have directed attention towards the paramount importance of sulphur compounds, especially in beer. These compounds originate from sulphur-containing amino acids. The sulphur compounds detected in beer include hydrogen sulphide, dimethyl and diethyl sulphides, methanethiol, diethyl disulphide and sulphur dioxide (DREWS *et al.*, 1969). Distilled beverages, too, contain sulphur compounds. Those found in raw grain spirit include hydrogen sulphide, methanethiol, carbon disulphide, dimethyl sulphide and dimethyl disulphide (Fig. 10) (RONKAINEN *et al.*, 1973).

Fatty acid esters make up the numerically largest aroma group produced by yeast. The amount of esters formed varies greatly from yeast to yeast. *Hansenula anomala* and *Candida krusei* yeasts produce less than *e.g. Schizosaccharomyces pombe* (PARFAIT *et al.*, 1972). Some years ago we found that the esterforming capacities of baker's yeast *(Saccharomyces cerevisiae)* and brewer's bottom yeast *(Saccharomyces uvarum)* are very different, with the former producing much more isoamyl acetate, ethyl caproate, ethyl caprylate and ethyl caprate (Fig. 11). The differences between yeasts become more apparent with increasing chain length of the acid moiety of the ester.

Yeast contains several esterases that can influence the formation or splitting of aroma esters. Esterases exist both inside and outside of the plasma membrane of the yeast cell, and more than half of them are located outside the membrane (SUOMALAINEN, 1981).

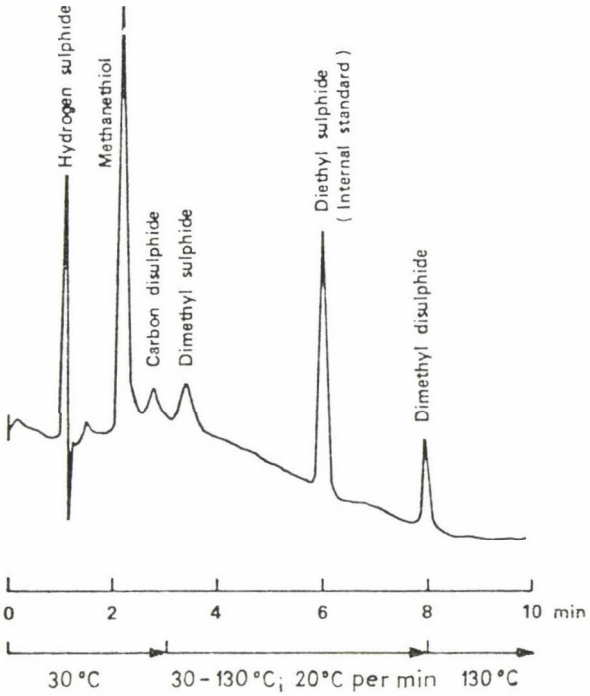


Fig. 10. Head-space chromatogram of sulfur compounds in raw grain spirit (RONKAINEN *et al.*, 1973)

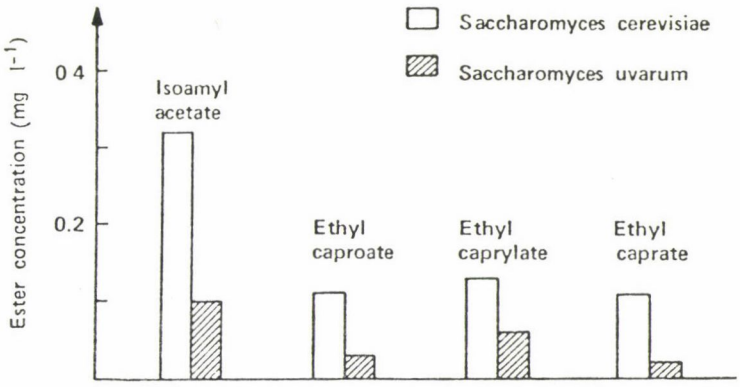


Fig. 11. The production of short chain fatty acid esters in fermentation with *Saccharomyces cerevisiae* and *S. uvarum* (NYKÄNEN, 1978)

In order to examine the hydrolysis ability of yeast esterases we chose ethyl caprylate as substrate because it is hydrolysed much more rapidly than, for example, isoamyl acetate, which, in fact, some esterases cannot hydrolyse at all (Fig. 12). With ethyl caprylate as substrate we found that the esterase

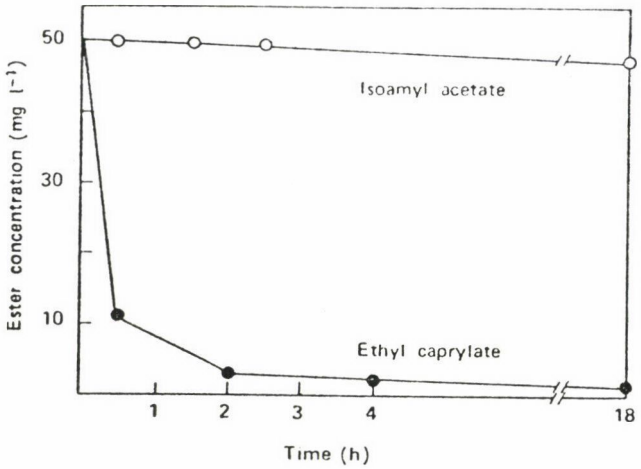


Fig. 12. Rate of hydrolysis of isoamyl acetate and ethyl caprylate during incubation of the esters with intact baker's yeast (PARKKINEN & SUOMALAINEN, 1982 a, b)

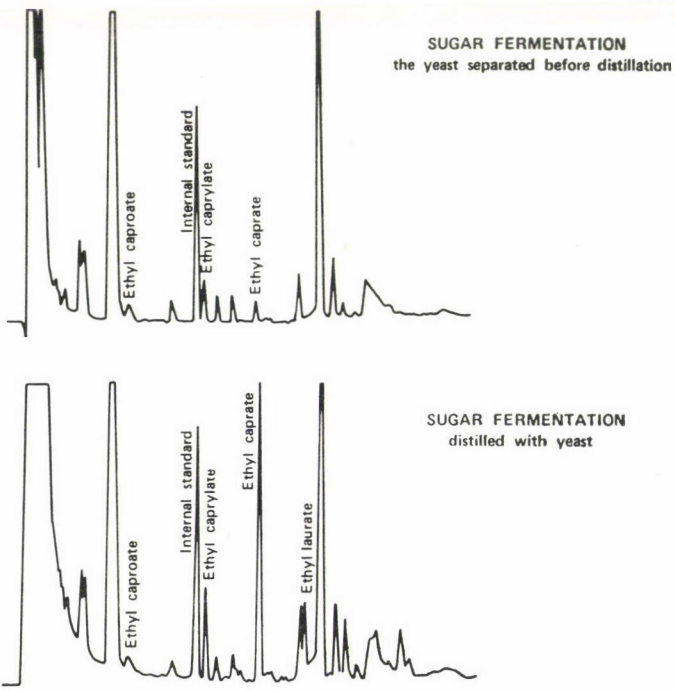


Fig. 13. Gas chromatograms of yeast-fermented products distilled before or after removal of yeast (NYKÄNEN *et al.*, 1977)

activity of intact yeast cells at pH 3 was only half of that at neutral pH (PARKKINEN & SUOMALAINEN, 1982 a, b). Thus hydrolysis occurs more slowly at acid pH.

The way in which yeast influences the ester content of a distilled beverage is not limited to the fermentation stage, it also makes a great difference as to whether or not the yeast is removed before distillation (NYKÄNEN *et al.*, 1977). When it remains in the medium to be distilled, the levels of the ethyl esters of caprylic, capric and lauric acids rise substantially (Fig. 13). The variable distribution of esters between the fermentation medium and the yeast cell is the reason for this phenomenon.

The distribution depends on the chain length of the acid moiety, *i.e.* on the lipophilic nature of the ester. The longer the chain length, the higher the proportion found inside the yeast cell. Isoamyl acetate, phenethyl acetate and ethyl caproate are found exclusively in the medium. Ethyl caprylate and ethyl caprate are to be found both in the cell and in the medium, whereas ethyl laurate is only found inside the cell. The type of yeast also has an effect on the distribution (NYKÄNEN & NYKÄNEN, 1977).

## Baking

The most important function of yeast in baking is to raise the dough through the action of the carbon dioxide formed during the fermentation of sugars in the dough (OURA *et al.*, 1980). Information about the sugar content of flours has long been available, but details had to wait for the development of chromatographic techniques. Some of the values thus collected for wheat flour are given in Table 1. An interesting point is that 60–75% of the free sugars in dough are fructose-containing sugars other than sucrose, *i.e.* glucofructosans and raffinose.

Table 1

*Free sugars in wheat flour, g per 100 g*

| Sugar | KOCH *et al.* (1951) | MACKENZIE (1958) | SAUNDERS *et al.* (1972) | SUOMALAINEN *et al.* (1972) |
|---|---|---|---|---|
| Glucose | 0.01 | 0.04 | 0.02 | 0.05 |
| Fructose | 0.02 | | 0.04 | 0.05 |
| Sucrose | 0.10 | 0.27 | 0.26 | 0.50 |
| Maltose | 0.07 | traces | 0.12 | 1.75 |
| Raffinose | 0.07 | 0.17 | traces | 0.45 |
| Glucofructosans | 0.18 | 0.78 | 1.38 | — |

The disappearance of sugars during the leavening of a straight dough has been followed with chromatography. The first clear change is the rapid consumption of sucrose: none can be detected after 1 hour's leavening (Fig. 14). Similarly, a dough initially containing 7.5% sucrose contains none after only 7 min. This is a consequence of the very active saccharase in baker's yeast, which hydrolyses sucrose much faster than yeast is able to utilise the hydrolysis products. The glucose content of dough falls rapidly so that after two hours the glucose concentration is almost zero.

Fructose is utilised in a somewhat different way. After an hour the level is the same as at the start, which means that an amount equivalent to that liberated in the hydrolysis of sucrose and raffinose has been consumed. Even after 3 hours some fructose remains in the dough. This preferential utilisation of glucose in dough has been frequently reported and the phenomenon has been shown to occur in laboratory fermentations as well (SUOMALAINEN & TOIVONEN, 1948).

The amount of maltose in dough increases throughout the fermentation period through the action of amylases, which start to function as soon as water is added to the flour. No maltose is consumed during the first hour of

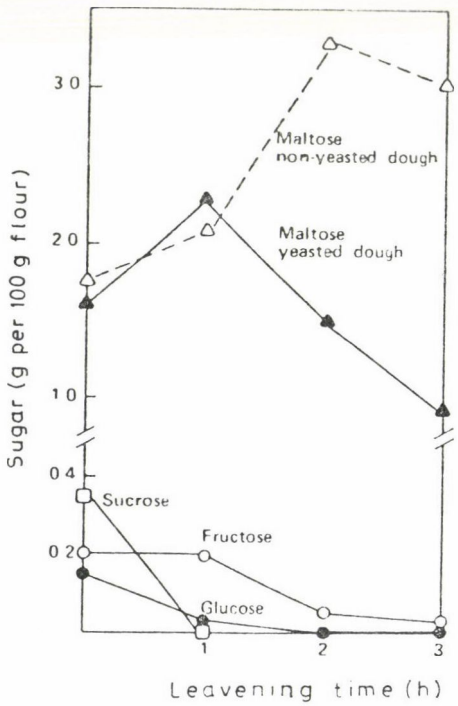


Fig. 14. Changes in the content of some free sugars in the dough during leavening, and of maltose in non-yeasted dough (---) (SUOMALAINEN *et al.*, 1972)

leavening, as is shown by the similar maltose contents in doughs with or without yeast (Fig. 14). After the first hour, however, the concentration of glucose and fructose is no longer at such a level as to sustain catabolite repression, so that α-glucosidase, which is present in most modern baker's yeasts as a constitutive enzyme, starts to hydrolyse maltose to glucose, which the yeast consumes immediately.

The annual consumption of fresh baker's yeast in Finland has been put at 2 kg per person, which as far as we know is the highest in the world. This is not because of illicit distillation (which certainly greatly increases yeast use in some countries), but it is due simply to the frequency of home baking. A third of all yeast sold is in 50 g packages of fresh yeast intended for home baking, and this is assumed to be the proportion actually used at home.

Rye bread and bread made from mixed flours including rye are so common in Finland that the rye consumption in bakeries and at home approaches half of that of wheat. In fact, rye bread, especially sour black bread (Fig. 15), is an extremely familiar item on Finnish dining tables. Various confectioneries are also very common leavened baking products, which, with their abundance of sugar and fat make their own demands on the type of yeast used. Of course,

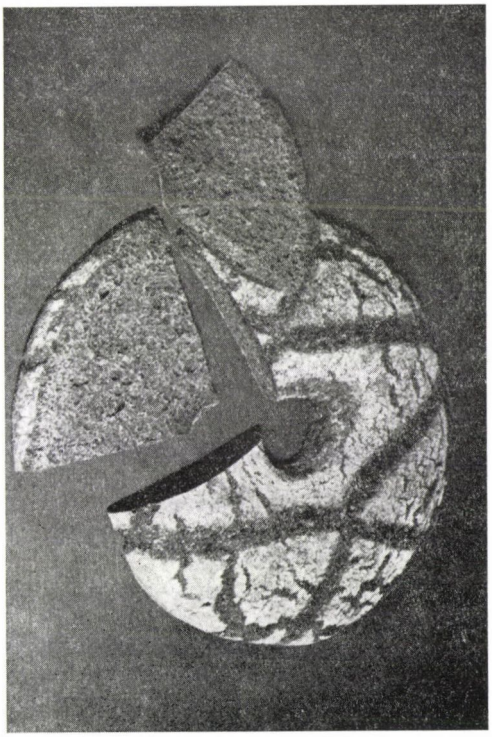

Fig. 15. Finnish rye bread

rye bread and lean white bread also require certain, very different characteristics of the yeast, so that when only one type of yeast is made the yeast factories must make a product that is simultaneously suited for these different applications. Consequently, Finnish yeast differs in some characteristics from those made on the continent or in Britain.

In 1963 the International Union of Pure and Applied Chemistry (IUPAC) charted the world production of baker's yeast for that year, and later revised and extended the values with figures four years later (IUPAC, 1966, 1971). The data, however, were far from complete, with the Soviet Union and other countries missing from Europe, the USA, Brazil and Uruguay being the only countries of the Western Hemisphere included, Africa was represented only by the Republic of South Africa, and Asia only by Japan.

The IUPAC statistics, despite being incomplete, have nevertheless been of value for later surveys. For instance, some of the information presented by PEPPLER (1978) is based on the IUPAC data. PEPPLER (1978) reports the baker's yeast production in dried tons which is roughly 1/4 of the fresh yeast tons used in the IUPAC statistics. BRONN (1976) in West-Berlin has extended the IUPAC data and concluded that the total production of fresh baker's yeast for 1974 was 970 000 tons. A more recent estimate made in ALKO (Table 2) amounts to 1 431 000 tons fresh yeast in 1980.

PEPPLER's figures for Africa clearly include only the Republic of South Africa and Zaire. Our most recent statistics for Africa, which may not be complete, give an annual production of fresh baker's yeast of 30 000–40 000 tons. The map in Fig. 16 shows the existing yeast factories in Africa and those

Table 2

*Estimated world production of baker's yeast in 1980 as tons fresh yeast (28—32% d.m.)* (EDELMANN, K., unpublished)

| | |
|---|---|
| Western Europe, EEC | 315 000 |
| Western Europe, remainder [a] | 128 000 |
| Eastern Europe [b] | 394 000 |
| North and Central America | 330 000 |
| South America | 75 000 |
| Africa | 47 000 |
| Asia | 132 000 |
| Australia | 10 000 |
| Total | 1 431 000 |

[a] Includes the Asiatic part of Turkey
[b] Includes the Asiatic Soviet Union

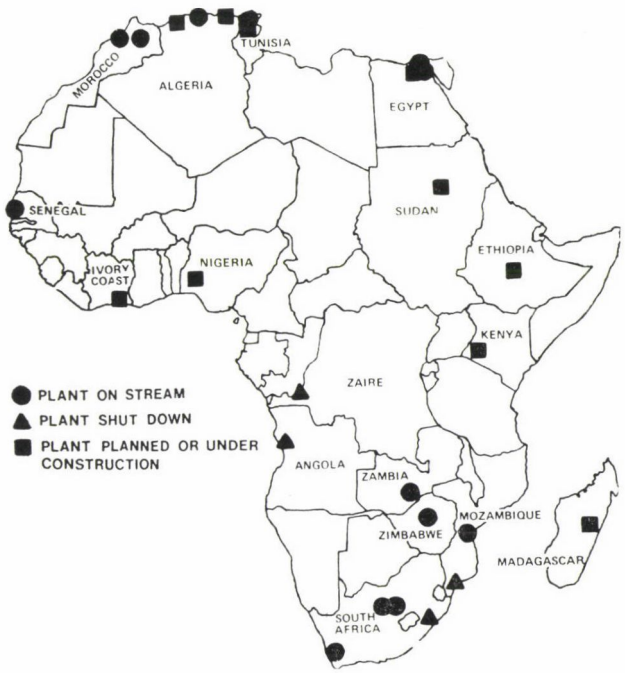


**Fig. 16. Baker's yeast plants in Africa 1980 (EDELMANN, K., unpublished)**

planned or under construction. In addition, some countries in the Middle East have shown interest in starting their own yeast production or expanding their capacity. However, it is impossible to predict which of these plans will be realised.

I hope that I have been successful in showing that yeast still continues to play a central role in fermentation industries and technical research.

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Address of the author:

Dr. Heikki SUOMALAINEN — Research Laboratories of the State Alcohol, Monopoly (Alko)
Box 350, SF-00101 Helsinki 10. Finland

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# DETERMINATION, BY HPLC, OF CAROTENOIDS IN PAPRIKA PRODUCTS

M. BARANYAI, Z. MATUS and J. SZABOLCS

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A HPLC method for quantitative determination of the individual carotenoids in paprika pigment has been developed. The determination consists of the following steps: extraction, saponification, HPLC separation, identification and quantitative evaluation of the carotenoids.

Using the HPLC system elaborated by LANGE (1976) we usually detected 16 carotenoids, of which 12 were identified by means of $t_R$ values, authentic samples and simple chemical reactions adapted for the HPLC technique.

For quantitative evaluation the band areas of a model solution containing the main carotenoids of paprika pigment were related to the band area of an internal canthaxanthin standard, which resulted in several empirical factors. In order to calculate the amount (mg) of a carotenoid, the related band area of the paprika sample to be determined, was multiplied by the respective factor.

Applications of the method to oleoresins, native and ground paprikas are presented in detail. In case of native paprika, the values (%) of the red components and the total carotenoid content (g $kg^{-1}$) agree with similar results obtained by the so-called "reduction method".

Determination of the pigment concentration in red paprika products was for long based on colorimetric (MOSTER & PRATER, 1952), then photometric and spectrophotometric measurements (BENEDEK, 1958; POHLE & GREGORY, 1960) of the total carotenoid content. Separation of the single carotenoids by column chromatography (CHOLNOKY *et al.*, 1955) and even by paper and thin-layer chromatography (VINKLER & KISZEL-RICHTER, 1972) proved too complicated for industrial purposes. The new "reduction method" (BARANYAI & SZABOLCS, 1976) enables the red and total (red + yellow) pigment contents to be determined without using any kind of chromatography, however, it fails to give enough information on the single carotenoids, which might be of great importance in agricultural and industrial research. The present paper describes how, besides industrial characterization of paprika products, the method of HPLC can be utilized in a kind of carotenoid analysis that meets the research worker's demands, too. Our method of determining carotenoid content in paprika products has been developed into a routine procedure maintaining the traditions of the past.

## 1. Materials and methods

### *1.1. Materials*

Analytical grade chemicals were used, and the sample of capsorubin (I) m.p. 190 °C; $\lambda_{max}(\varepsilon_{max})$ in benzene: 522 (119 100), 487 (129 800) and 463 (89 200) nm, capsanthin epoxide (II) m.p. 185 °C; $\lambda_{max}(\varepsilon_{max})$ in benzene: 508 (80 800), 479 (99 800) and 454 (88 300) nm, violaxanthin (III) m.p. 170 °C; $\lambda_{max}$ ($\varepsilon_{max}$) in benzene: 483 (130 700), 453 (135 400) and 426 (88 900) nm, capsanthin (IV) m.p. 161 °C; $\lambda_{max}$ ($\varepsilon_{max}$) in benzene: 486 (113 200) nm, antheraxanthin (V) m.p. 176 °C; $\lambda_{max}$ ($\varepsilon_{max}$) in benzene: 488 (113 600), 458 (127 500) and 434 (86 500) nm, mutatoxanthin (VI) m.p. 168 °C; $\lambda_{max}$ in benzene: 465, 438 and 414 nm, zeaxanthin (VII) m.p. 204 °C; $\lambda_{max}$ ($\varepsilon_{max}$) in benzene: 493 (107 300) and 463 (124 200) nm, cryptocapsin (VIII) m.p. 162 °C; $\lambda_{max}$ ($\varepsilon_{max}$) in benzene: 486 (111 900) nm, $\beta$-cryptoxanthin (IX) m.p. 157 °C; $\lambda_{max}$ ($\varepsilon_{max}$) in benzene: 493 (111 400) and 463 (126 700) nm, $\beta$-carotene (X) m.p. 174 °C; $\lambda_{max}$ ($\varepsilon_{max}$) in benzene: 494 (106 100) and 464 (122 300) nm, capsorubol (XII) $\lambda_{max}$ in benzene: 466, 445 and 421 nm, capsanthol (XIV) m.p. 171 °C; $\lambda_{max}$ ($\varepsilon_{max}$) in benzene: 487 (120 300), 457 (128 200) and 434 (94 000) nm, cryptocapsol (XV) $\lambda_{max}$ in benzene: 487, 457 and 432 nm, and auroxanthin (XVIII) $\lambda_{max}$ in benzene: 438, 410 and 386 nm were taken from our collection. Canthaxanthin (XI) [*Hoffmann-La Roche*, m.p. 205 °C, recrystallized from benzene-petroleum ether (b.p. 30–40 °C)] was used as an internal standard [m.p. 206 °C; $\lambda_{max}$ ($\varepsilon_{max}$) in benzene: 483 (117 300) nm]. The acetonic model and stock solutions were stored under nitrogen, away from light at –20 °C.

### *1.2. Methods*

The extraction was carried out according to BENEDEK (1958) and 20–40 $cm^3$ of the benzene solution obtained (100 $cm^3$) was evaporated in vacuo at 45 °C. The residue was dissolved in 50 $cm^3$ of ether and allowed to stand in the presence of 5 $cm^3$ of 30% methanolic KOH solution under nitrogen, at room temperature for 16 hrs. After completion of the saponification, the two phases were poured into a separatory funnel, diluted with ether and some water to transfer all the saponified carotenoids into the ethereal phase. (Autooxidation products cannot be transferred from the KOH-methanol-water phase into the ethereal phase.) Then washed with water free from alkali, dried over anhydrous $Na_2SO_4$, filtered off and mixed with a stock solution of canthaxanthin (2 mg of canthaxanthin in 100 $cm^3$ benzene), evaporated in vacuo at 35 °C and the residue was dissolved in 1–2 $cm^3$ of acetone for HPLC. Starting from oleoresins, 20–40 $cm^3$ of an about 0.5% benzene solution was saponified under the same conditions. $NaBH_4$ reduction and acid treatment were performed according to MATUS and co-workers (1981).

*1.3. Instruments*

The light absorption spectra were run on a *Perkin-Elmer* Model 402 spectrophotometer. For HPLC analysis a *Liquochrom* Model 307 (Labor MIM, Hungary) liquid chromatograph, a stop-flow injector (Labor MIM), a *Carl-Zeiss* recorder (type K 201), a home-built UV detector (OHMACHT, 1979a) and a home-made mixing chamber (OHMACHT, 1979b) were used. This work was carried out using a reverse phase with a particle size of 10 $\mu$m and gradient elution (LANGE, 1976) unless otherwise stated. The samples were injected as acetonic solutions.

Column: glass-lined metal column, 200×4 mm *i.d.*, packed with *Nucleosil* 10 $C_{18}$ (MACHEREY-NAGEL) by the balanced viscosity method; flow rate: 1.31 $cm^3$ $min^{-1}$ (80 bar) after the gradient 1.43 $cm^3$ $min^{-1}$ (40 bar); eluant: 100 : 40 (v/v) acetone–water then changed to 100 : 5; quantitity injected: 5–10 $mm^3$ corresponding to 0.05–0.8 $\mu$g of the individual carotenoids; monitoring wavelengths: 510, 480, 450, 428, 402 and 340 nm.

## 2. Results

The determination is based on the HPLC-system elaborated by LANGE (1976) and our own method worked out for detecting various types of carotenoids by HPLC (MATUS *et al.*, 1981). Methodologically the determination consists of the following four steps: extraction, saponification, HPLC separation and quantitative evaluation of chromatograms using canthaxanthin as an internal standard.

*2.1. Extraction*

Ground paprika was extracted by the traditional method (BENEDEK, 1958) to enable a comparison of the data obtained by HPLC analysis with the *Benedek* numbers.

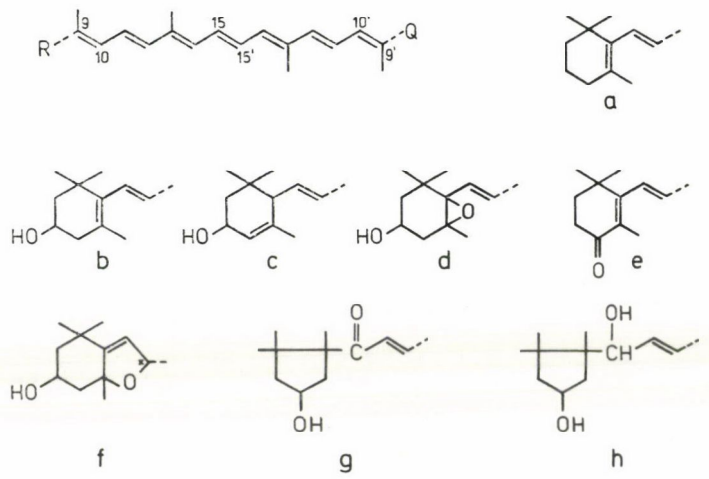
*2.2. Saponification*

Before HPLC separation the epiphasic carotenoid esters must be saponified to increase the differences in polarity between the single components of the carotenoid extract. Both heterogeneous and homogeneous (DAVIES, 1976) saponification can be applied. Owing to the autooxidation products formed during either industrial processes or storage, some loss of the total carotenoid content (differences in the *Benedek* numbers before and after saponification) resulted from the saponification. It should, however, be emphasized that saponification of an extract obtained from native paprika does not cause any loss of carotenoids (Table 2).

Table 1

*The structure of identified paprika carotenoids and some of their derivatives*

Capsorubin (I): R = Q = g; Capsanthin epoxide (II): R = d, Q = g; Violaxanthin (III): R = Q = d; Capsanthin (IV): R = b, Q = g; Antheraxanthin (V): R = b, Q = d; Mutatoxanthin (VI): R = b, Q = f; Zeaxanthin (VII): R = Q = b; Cryptocapsin (VIII): R = a, Q = g; $\beta$-Cryptoxanthin (IX): R = a, Q = b; $\beta$-Carotene (X): R = Q = a; Canthaxanthin (XI): R = Q = e; Capsorubol (XII): R = Q = h; Capsanthol epoxide (XIII): R = d, Q = h; Capsanthol (XIV): R = b, Q = h; Cryptocapsol (XV): R = a, Q = h; Capsochrom (XVI): R = f, Q = g; Capsochromol (XVII): R = f, Q = h; Auroxanthin (XVIII): R = Q = f; Lutein (XIX): R = b, Q = c.




## *2.3. Identification of the carotenoids*

The bands of carotenoids in a chromatogram were identified by means of simple chemical reactions (MATUS *et al.*, 1981) and the retention times using authentic samples and appropriate variation of the wavelengths of detection. The findings of earlier separations of paprika pigments based on column, thin-layer or paper chromatography (SCHNEIDER, 1977) were also taken into consideration. In general, the chromatograms in HPLC were performed at 480 nm where the great majority of genuine paprika pigments have considerable absorbance.

Figures 1a and 1b show the chromatograms obtained at 510, 480 and 428 nm of a paprika (*Capsicum annuum lycopersiciforme rubrum*, Pécs, 1980) extract. A comparison of the chromatograms reveals that bands 1, 2, 5, 6 and 13 represent polyene ketones with conjugated oxo groups, which still show relatively strong absorption at 510 nm (Fig. 1a) and, in contrast, relatively moderate absorption at 428 nm (Fig. 1b). The conjugated oxo groups of these pigments were confirmed by $NaBH_4$ reduction of the original extract, which resulted in a chromatogram (Fig. 2) with a pronounced change of the $t_R$ values of bands

Table 2

*Determination of the red and total pigment content in oleoresins[a] (OR), ground paprika[a] (GP) and native, ripe paprika[b] (P) samples using different methods*

| Sample | Color index[c] ($10^{-3}$) | *Benedek* number | | Reduction method | | HPLC method | |
|---|---|---|---|---|---|---|---|
| | | Extract (g kg$^{-1}$) | Saponified extract (g kg$^{-1}$) | Total pigment (g kg$^{-1}$) | Red components (%) | Total pigment (g kg$^{-1}$) | Red components (%) |
| OR 1 | 20 | 14.2 | 12.6 | 15.7 | 68.8 | 12.5 | 72.9 |
| OR 2 | 40 | 26.6 | 24.0 | 29.0 | 69.2 | 26.6 | 74.5 |
| OR 3 | 60 | 46.7 | 40.6 | 49.8 | 67.4 | 36.9 | 71.5 |
| OR 4 | 80 | 53.1 | 48.7 | 56.9 | 68.6 | 48.3 | 72.4 |
| OR 5 | 100 | 75.2 | 66.5 | 82.0 | 68.6 | 66.4 | 72.2 |
| OR 6 | 40 | 26.2 | 23.1 | 28.3 | 75.0 | 23.6 | 69.4 |
| OR 7 | 60 | 41.9 | 37.5 | 44.6 | 68.0 | 38.8 | 69.2 |
| OR 8 | 80 | 56.0 | 50.2 | 61.6 | 68.2 | 52.4 | 67.4 |
| OR 9 | 100 | 63.8 | 58.9 | 68.0 | 60.0 | 63.5 | 64.5 |
| GP 1 | — | 7.2 | 6.5 | 6.9 | 66.7 | 6.91 | 64.1 |
| GP 2 | — | 3.4 | 3.1 | 3.4 | 59.4 | 3.06 | 59.7 |
| P 1 | — | 17.6 | 17.3 | 19.0 | 54.3 | 16.5 | 56.1 |

[a] Commercial products
[b] *Capsicum annuum lycopersiciforme rubrum*
[c] Results of quality control in the factory

1, 2, 5, 6 and 13. It can be seen, moreover, that bands 6 and 8 cover bands 7' and 8' (Fig. 2). Since a repeated $NaBH_4$ reduction of the already reduced extract has no effect on the $t_R$ values, it is obvious that the pigments in bands 7' and 8' are genuine non-oxo carotenoids, and do not result from an incomplete reduction.

Then the original extract was treated with dilute acid (MATUS *et al.*, 1981) to convert 5,6-epoxides into furanoid oxides, which have different $\lambda_{max}$-a and $t_R$v alues. Figure 3 demonstrates that the pigments present in bands 2 and 4 have 5,6-epoxy character. Figure 4 presents a more informative chromatogram produced by a combination of acid treatment (epoxy group test) and $NaBH_4$ reduction (oxo group test) showing that the pigments of bands 2, 7 and 8 are also 5,6-epoxy carotenoids. On setting detection at 428 nm (Fig. 5), bands 2', 6', 7', 8' and 9' can be identified as furanoid oxide derivatives formed from 5,6-epoxides. One of them (band 9') is present in traces in the original extract as seen in Fig. 1b (band 9).

In summary, the paprika carotenoids (Fig. 1) can be divided into 4 groups: a) pigments of oxo and 5,6-epoxy character (band 2); b) pigments of oxo character (bands 1, 5, 6, 7, 8, 13); c) pigments of 5,6-epoxy character (bands 4, 6, 8);

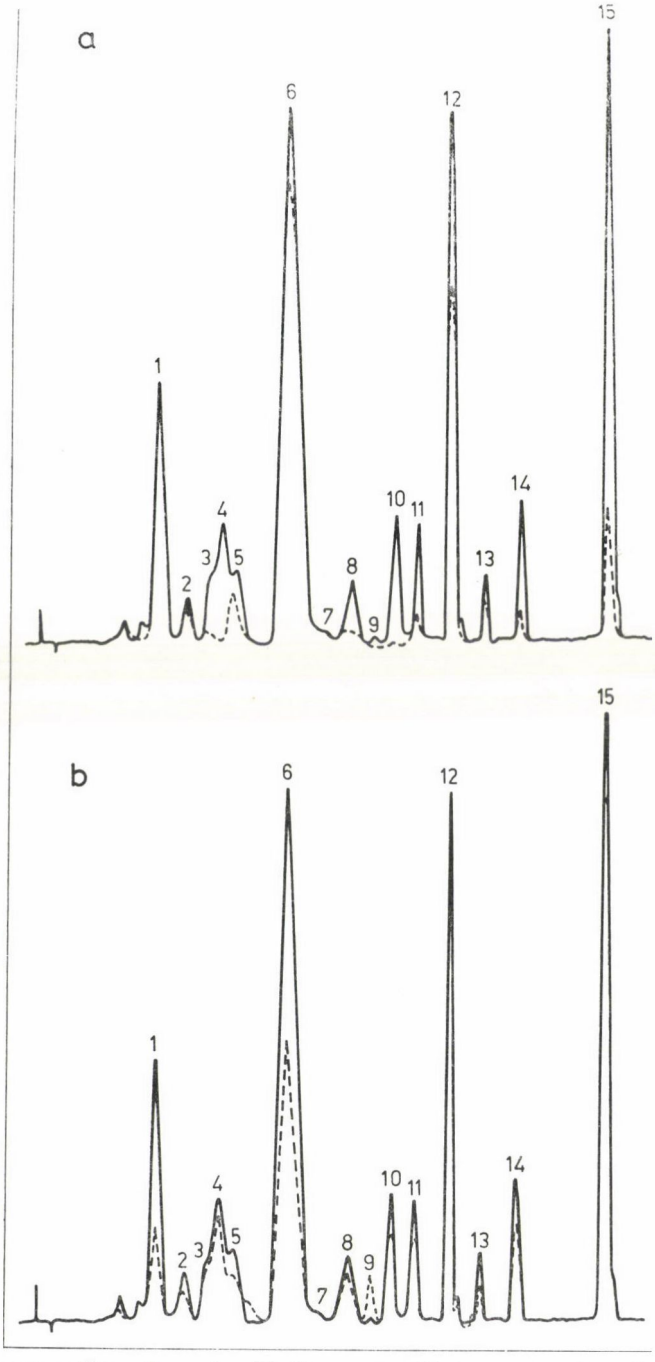



Fig. 1. HPLC separation of carotenoids in a saponified extract of native paprika (P). Fig. 1a.: detection at 480 (——) and 510 (- - - -) nm; Fig. 1b.: detection at 480 (——) and 428 (- - - -) nm. Column and conditions as in text. Bands: 1 = I, 2 = II, 3 = "Pigment-1", 4 = III, 5 = "Pigment-2", 6 = IV + "Pigment-3", 7 = IV (9-*cis*), 8 = V + + IV (13-*cis*), 9 = VI, 10 = "Pigment-4", 11 = VII, 12 = XI, 13 = VIII, 14 = IX, 15 = X

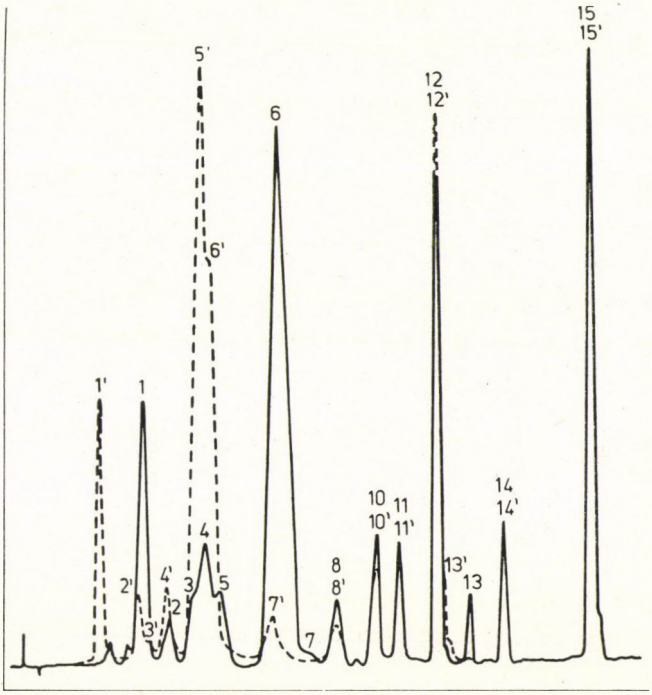



Fig. 2. Chromatograms before (———) and after (- - - -) $NaBH_4$ reduction; detection a 480 nm. Column and conditions as in text. Bands: 1 = I, 2 = II, 3 = "Pigment-1" 4 = III, 5 = "Pigment-2", 6 = IV + "Pigment-3", 7 = IV (9-*cis*), 8 = V + IV (13-*cis*), 9 = VI, 10 = "Pigment-4", 11 = VII, 12 = XI, 13 = VIII, 14 = IX, 15 = X. Bands ('): 1' = XII, 2' = XIII, 3' (unidentified), 4' = reduced "Pigment-2", 5' = XIV (epimer) + "Pigment-1", 6' = XIV (epimer) + III, 7' = "Pigment-3", 8' = V, 9' = = 9 = VI, 10' = 10 = "Pigment-4", 11' = 11 = VII, 12' = 12 = XI, 13' = XV, 14' = 14 = IX, 15' = 15 = X

and pigments showing no oxo or epoxy character (bands 3, 9, 10, 11, 14, 15). Furthermore, on comparing the retention times and the mixed chromatograms with authentic samples, the following carotenoids (80–85 p.c. of the total pigment content) were identified: capsorubin (I), capsanthin epoxide (II), violaxanthin (III), capsanthin (IV), 9-*cis*-capsanthin (IV), 13-*cis*-capsanthin (IV), antheraxanthin (V), mutatoxanthin (VI), zeaxanthin (VII), cryptocapsin (VIII), $\beta$-cryptoxanthin (IX) and $\beta$-carotene (X). Although we failed to separate zeaxanthin (VII) from lutein (XIX) in this system, using another HPLC system (*Partisil*-10 $\mu$, 1.5% iso-propanolic benzene and an authentic sample of lutein originated from *Viola* tricolor) we succeeded in showing that lutein (XIX) did not occur in the native paprika sample investigated.

Finally, it should be mentioned that in order to clarify their exact structure isolation, on a preparative scale, of the pigments in bands 3, 5 and 10 (Fig 1: "Pigment-1", "Pigment-2", "Pigment-4") and in band 7' (Fig. 2: "Pigment-3") is in progress.

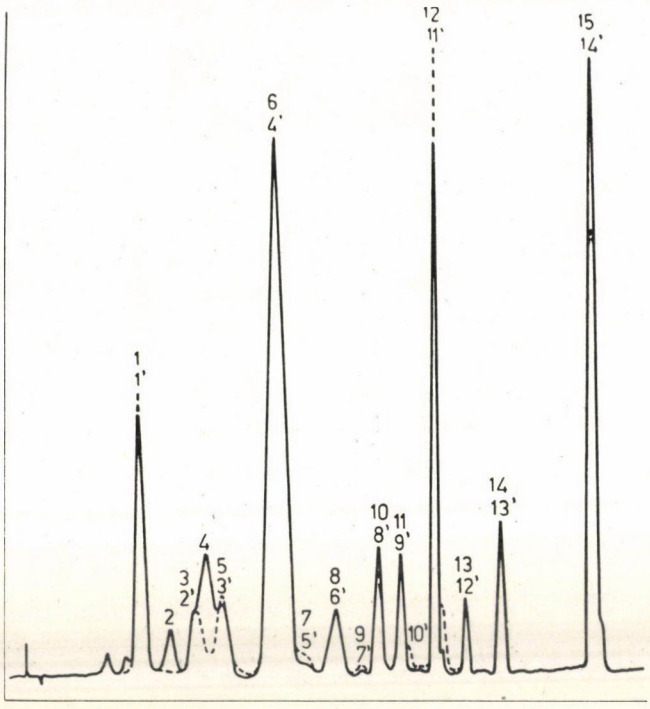



Fig. 3. Chromatograms before (———) and after (- - - -) acid treatment; detection at 480 nm. Column and conditions as in text. Bands: 1 = I, 2 = II, 3 = "Pigment-1", 4 = III, 5 = "Pigment-2", 6 = IV + "Pigment-3", 7 = IV (9-*cis*), 8 = V + IV (13-*cis*), 9 = VI, 10 = "Pigment-4", 11 = VII, 12 = XI, 13 = VIII, 14 = IX, 15 = X. Bands ('): 1' = 1 = I, 2' = 3 = "Pigment-1", 3' = 5 = "Pigment-2", 4' = 6 = IV + + "Pigment-3", 5' = 7 = IV (9-*cis*), 6' = IV (13-*cis*), 7' = 9 = VI, 8' = 10 = "Pigment-4", 9' = 11 = VII, 10' = VII (13-*cis*), 11' = 12 = XI, 12' = 13 = VIII, 13' = = 14 = IX, 14' = 15 = X

### *2.4. Quantitative evaluation*

The chromatograms were evaluated quantitatively by relating the band areas of the individual carotenoids to that of canthaxanthin (XI) used as an internal standard. Canthaxanthin has certain useful properties: it is a synthetic, commercially available carotenoid, the stock solution can be kept for weeks and its band does not interfere with others.

*2.4.1. Calibration.* From the main components of paprika pigment (I, IV, VII, X) a stock solution of $10^{-4}$ M and from the internal standard of canthaxanthin another stock solution of $10^{-4}$ M were prepared in benzene. Aliquot volumes of the two stock solutions were mixed to obtain a series of model solutions with different concentrations. Before injection they were evaporated in vacuo to dryness and dissolved in acetone. After HPLC separation, the proportions between the band areas of the individual carotenoids and that

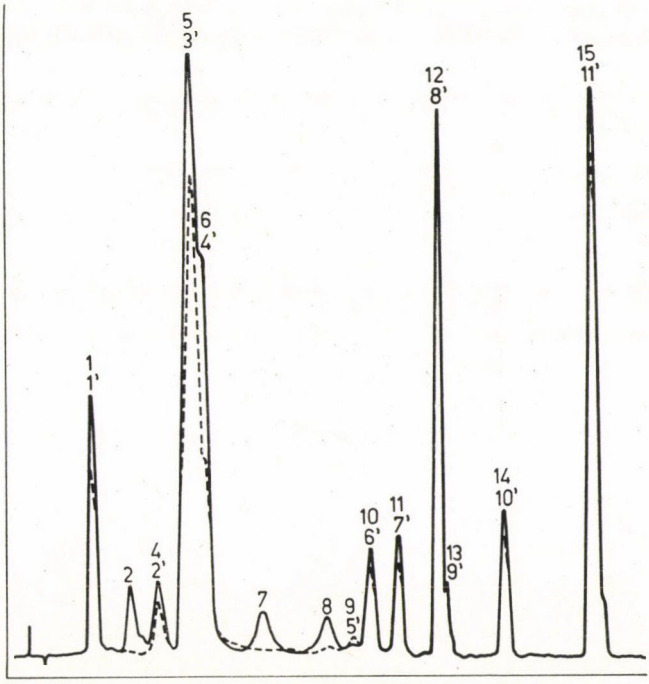



Fig. 4. Chromatograms after $NaBH_4$ reduction (———) and after acid and $NaBH_4$ treatment (- - - -); detection at 480 nm. Column and conditions as in text. Bands: 1 = = XII, 2 = XIII, 3(unidentified), 4 = reduced "Pigment-2", 5 = XIV (epimer) + + "Pigment-1", 6 = III + XIV (epimer), 7 = "Pigment-3", 8 = V, 9 = VI, 10 = "Pigment-4", 11 = VII, 12 = XI, 13 = XV, 14 = IX, 15 = X. Bands('): 1' = 1 = XII, 2' = 4 = reduced "Pigment-2", 3' = 5 = XIV (epimer) + "Pigment-1", 4' = XIV (epimer), 5' = 9 = VI, 6' = 10 = "Pigment-4", 7' = 11 = VII, 8' = 12 = XI, 9' = = 13 = XV, 10' = 14 = IX, 11' = 15 = X

of the internal canthaxanthin standard were plotted against the proportions between the amounts (mg) of the carotenoids and that (mg) of the internal canthaxanthin standard. As seen in Fig. 6, *e.g.*, the curve of capsanthin shows a linear relationship with the internal standard. A similar relationship was found for all components of the model solutions. tg $\alpha$ of the calibration curves serves as factor by which the band area (in relation to the band area of the internal canthaxanthin standard) is multiplied in order to obtain the amount (mg) of a certain carotenoid.

Since 9-*cis*-capsanthin (IV) and 13-*cis*-capsanthin (IV) belong to the red components of paprika pigment (Table 3), calibration for *cis* isomers is also warranted. Although a particular calibration curve for each *cis* isomer has not been drawn, factors can be calculated for *cis* isomers, too. As the mol-extinction values (in benzene) of all-*trans*-capsanthin (IV) (113 000), 9-*cis*--capsanthin (91 000) and 13-*cis*-capsanthin (79 000) are known, the factors for

Table 3

*Quantitative distribution of carotenoids in oleoresin[a] (OR) and ground paprika[a] (GP) samples as determined by the HPLC method*

| Sample | Total pigment (g kg$^{-1}$) | Percentage of total pigment | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | | Capsorubin | Violaxanthin[b] | Capsanthin | Zeaxanthin | Cryptocapsin | β-Cryptoxanthin | β-Carotene |
| OR 1 | 12.5 | 4.56 | 3.13 | 67.76 | 14.00 | 0.60 | 4.00 | 6.32 |
| OR 2 | 26.6 | 5.30 | 3.76 | 68.74 | 12.62 | 0.49 | 3.96 | 5.15 |
| OR 3 | 36.9 | 4.25 | 3.33 | 66.56 | 13.50 | 0.68 | 4.85 | 6.78 |
| OR 4 | 48.3 | 4.91 | 2.84 | 66.85 | 13.40 | 0.66 | 4.49 | 6.81 |
| OR 5 | 66.4 | 5.62 | 3.46 | 66.04 | 13.55 | 0.56 | 4.25 | 6.52 |
| OR 6 | 23.6 | 7.76 | 2.54 | 59.45 | 11.45 | 2.07 | 5.89 | 10.81 |
| OR 7 | 38.8 | 7.60 | 2.81 | 59.74 | 11.89 | 1.88 | 5.59 | 10.51 |
| OR 8 | 52.4 | 7.61 | 3.21 | 57.78 | 12.60 | 2.04 | 5.84 | 10.95 |
| OR 9 | 63.5 | 4.30 | 3.17 | 58.85 | 16.12 | 1.31 | 5.37 | 10.90 |
| GP 1 | 6.04 | 5.46 | 6.62 | 54.80 | 24.25 | 0.41 | 3.89 | 4.55 |
| GP 2 | 3.06 | 5.23 | 5.23 | 57.51 | 18.95 | 0.98 | 5.88 | 6.21 |

[a] Commercial products.
[b] Mixed with unidentified pigments.

*cis* isomers can be calculated as followes:

$$\text{factor}_{cis} = \frac{\text{factor}_{trans} \times \varepsilon_{trans}}{\varepsilon_{cis}}$$

The factors for all-*trans*, 9-*cis* and 13-*cis* capsanthins are 1.07, 1.33 and 1.53

On the other hand, as has been pointed out before (BARANYAI & SZABOLCS, 1976) the isomeric mixture of the individual carotenoids in stored paprika products and the isomeric equilibrium mixtures formed by iodine are of about the same composition, the amount (mg) of capsanthin and its 9-*cis* and 13-*cis* isomers can be calculated together by a factor obtained not from the plot of capsanthin (IV) and the internal canthaxanthin (XI) standard, but from the iodine-isomerized equilibrium mixture of capsanthin and the internal canthaxanthin standard. As is seen in Fig. 6, and according to expectations, the factor of the iodine-isomerized equilibrium mixture is higher than that of capsanthin. In the presence of *trans*- and *cis*-capsanthins, therefore, the band areas (related to the internal standard) of the *trans*- and *cis*-capsanthins are added and then multiplied by a factor obtained for the iodine-isomerized equilibrium mixture of capsanthin. This is the only way to calculate when the *cis* isomers do not separate from the all-*trans* forms (*e.g.* β-cryptoxanthin set

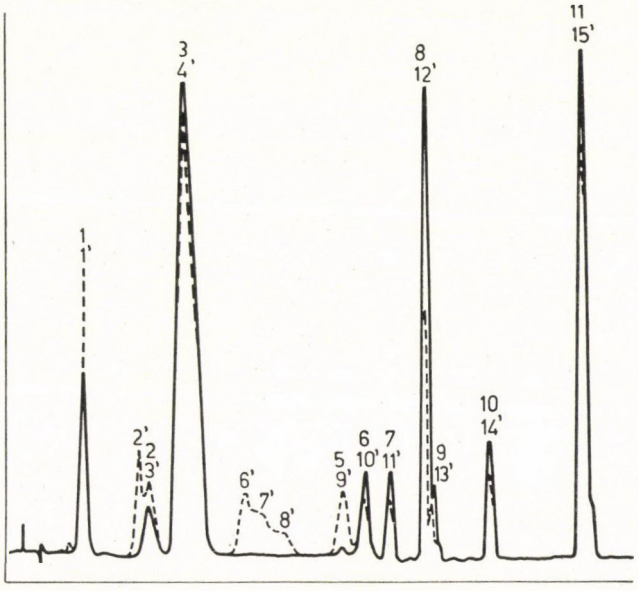



Fig. 5. Chromatograms after acid and $NaBH_4$ treatment; detection at 480 (———) and 428 (- - - -) nm. Column and conditions as in text. Bands: 1 = XII, 2 = reduced "Pigment-2", 3 = XIV (epimer) + "Pigment-1", 4 = XIV (epimer), 5 = VI, 6 = "Pigment-4", 7 = VII, 8 = XI, 9 = XV, 10 = IX, 11 = X. Bands('): 1' = 1 = XII, 2' = XVII, 3' = 2 = reduced "Pigment-2", 4' = XIV (epimer) + "Pigment-1", 5' = = 4 = XIV (epimer), 6' = XVIII (epimer), 7' = XVIII (epimer), 8' (unidentified), 9' = 5 = VI, 10' = 6 = "Pigment-4", 11' = 7 = VII, 12' = 8 = XI, 13' = 9 = XV, 14' = 10 = IX, 15' = 11 = X

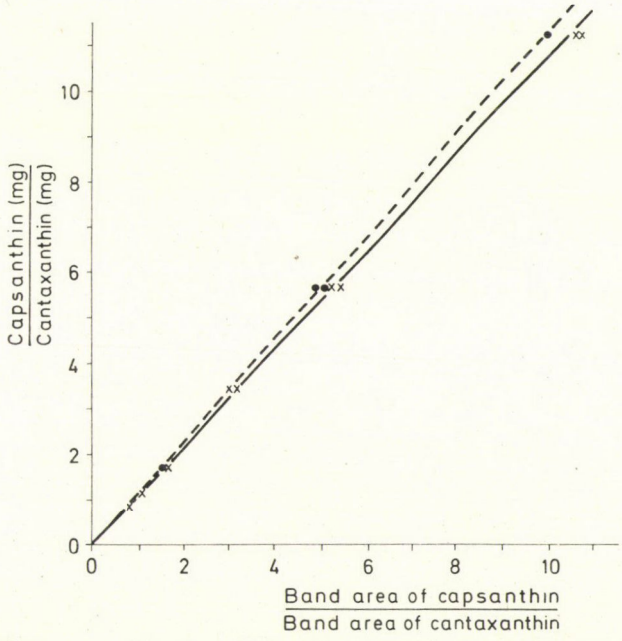



Fig. 6. Calibration curve for capsanthin (IV) determination. Empirical factors of the major carotenoids: capsorubin (I) = 0.96; capsanthin (IV) = 1.07; zeaxanthin (VII) = = 1.16; $\beta$-carotene (X) = 1.15. The factors of other carotenoids were extrapolated

and β-carotene set). Although these average values certainly meet most of the industrial demands, the correctness of the calculation depends on the composition of paprika products.

*2.4.2. Application of the method.* Application of the method to oleoresins (OR), native (P) and ground paprikas (GP) are demonstrated in Figs. 1, 7 and Tables 2, 3, 4. As has been stated, in the case of native paprika (Fig. 1), 16 bands were detected, of which the following were found in the oleoresins (Fig. 6): capsorubin (I), 9-*cis*-capsorubin, 13-*cis*-capsorubin, violaxanthin (III), capsanthin (IV), 9-*cis*-capsanthin, 13-*cis*-capsanthin, "Pigment-4", zeaxanthin (VII), *cis*-zeaxanthin, cryptocapsin (VIII), β-cryptoxanthin (IX) and β-carotene (X). In this system zeaxanthin (VII) cannot be separated from lutein (XIX), and *cis* β-cryptoxanthins and *cis* β-carotenes from the corresponding all-*trans* forms (IX and X). The so-called "Pigment-4" detected in native paprika is also present in oleoresins (band 8). A comparison of the chromatograms of an oleoresin sample (Fig. 7) and that of a native paprika (Fig. 1a) shows that only minor bands, between capsanthin (band 5) and 9-*cis*-capsorubin (band 2), amounting to 2.5–3.5% of the total pigment content, became

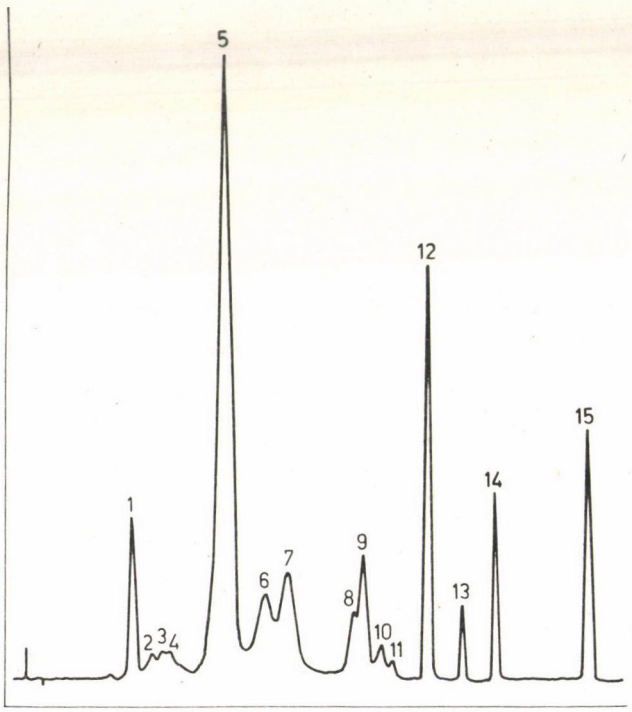



Fig. 7. HPLC separation of carotenoids in a saponified extract of oleoresin (OR). Column and conditions as in text; detection at 480 nm. Bands: 1 = I, 2 = I (9-*cis*), 3 = I (13-*cis*), 4 = III + unidentified, 5 = IV, 6 = IV (9-*cis*), 7 = IV (13-*cis*), 8 = "Pigment-4", 9 = VII, 10 = VII (9-*cis*), 11 = VII (13-*cis*), 12 = XI, 13 = VIII, 14 = IX, 15 = X

indistinctly broadened as a result of some degradation products. However, it was proved that this broad band (3–4) contained a small amount of 13-*cis*-capsorubin, violaxanthin, "Pigment-2" with an oxo group and "Pigment-1" without oxo and epoxy groups.

The band shapes are suitable for quantitative measurements, which are presented in Table 3. In addition, Table 2 shows the red components (g $kg^{-1}$), the total carotenoid content (g $kg^{-1}$), both determined by HPLC as well as the reduction method (BARANYAI & SZABOLCS, 1976), and the *Benedek* numbers after and before saponification. As is seen, in contrast with the results of native paprika, the total carotenoid contents are always lower with HPLC than those obtained with the reduction method. The proportion of the two values agrees with the proportion of the *Benedek* numbers determined after and before saponification. This is so because HPLC was carried out from the saponified extract and the 'reduction method' from the extract itself. The differences in the percentage of red components obtained with the HPLC method and the 'reduction method' might result from the loss of carotenoids due to saponification.

## 3. Conclusions

A quantitative determination by HPLC was developed for carotenoids in native paprika and paprika products. The method is recommended for agricultural research and industrial purposes. The steps of the method and our observations are as follows:

(a) Standard extraction, measuring the *Benedek* number.

(b) Saponification, either in a heterogeneous (16 hrs at 20 °C) or in a homogeneous (1 hr at 50 °C) phase.

The amount of degraded carotenoids present in commercial paprika products, which manifests itself as loss of saponification, was measured as the difference between the *Benedek* numbers before and after saponification. When starting from native paprika, there is no loss of saponification.

(c) Using a reverse phase and a gradient elution technique, 14–16 bands were detected. It should be noted that capsanthin epoxide (II) was also identified in a native, ripe sample of *Capsicum annuum lycopersiciforme rubrum* whose occurrence (CURL, 1962; DAVIES *et al.*, 1970) was questioned by us earlier (BARANYAI *et al.*, 1977). A red component ("Pigment-2") and three yellow components ("Pigment-1", "Pigment-3" and "Pigment-4") could not be identified as paprika carotenoids already known.

(d) Quantitative evaluation, using canthaxanthin (XI) as an internal standard, is possible. Calibration factors for the all-*trans* and several *cis* components were determined by means of model solutions. The reproducibility of the quantitative determination gave satisfactory results listed in Table 4.

Table 4

*Quantitative distribution of carotenoids in a native, ripe sample of* Capsicum annuum lycopersiciforme rubrum *as determined by the HPLC method; the table also indicates standard deviations*[a]

| Carotenoid | Pigment content (g kg$^{-1}$) | Percentage of total pigment (%) | Standard deviation (g kg$^{-1}$) |
|---|---|---|---|
| Capsorubin | 37.45 | 9.54 | 1.25 |
| Capsanthin epoxide | 10.15 | 2.59 | 0.52 |
| Violaxanthin and "Pigment-1" | 30.98 | 7.89 | 2.32 |
| "Pigment-2" | 15.93 | 4.06 | 2.04 |
| Capsanthin and "Pigment-3" | 149.35 | 38.09 | 3.68 |
| Antheraxanthin and 13-*cis*-capsanthin | 19.73 | 5.02 | 1.40 |
| "Pigment-4" | 16.44 | 4.19 | 0.84 |
| Zeaxanthin | 15.70 | 4.00 | 0.31 |
| Cryptocapsin | 7.07 | 1.80 | 0.54 |
| $\beta$-Cryptoxanthin | 16.56 | 4.22 | 0.45 |
| $\beta$-Carotene | 73.04 | 18.60 | 1.84 |
| Total | 392.2 | 100.00 | 9.56 |
| Red components | 220.1 | 56.1 | 4.41 |

[a] The data of nine chromatograms obtained from three separate saponifications were used or calculation

(e) The data of several paprika analyses obtained by HPLC as well as the 'reduction method' were compared to the *Benedek* numbers.

*

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Address of the authors:

Dr. Márta BARANYAI
Zoltán MATUS
Dr. József SZABOLCS*

Institute of Chemistry,
University Medical School
H-7643 Pécs, Szigeti út 12. Hungary

* Author to whom correspondence should be addressed.

# BOOK REVIEW

JAMES, W. P. T. & THEANDER, O.:

## The analysis of dietary fiber in food

(Marcel Dekker Inc., New York and Basel, 1980; 276 pages)

Dietary fiber is defined as the sum of polysaccharides and lignin in foods which are resistant to the enzymes of the human digestive tract. They are known to have different physiological effects.

In the course of the last few years, there has been a great number of investigators making efforts to determine dietary fiber in foodstuffs using different methods based on different theories. It has become inevitable to come to some agreement as to the most appropriate approach to the analysis.

In December 1977, at a meeting (in Lyon, France) sponsored by the Committee on Medical Research of the European Economic Community and by the International Agency for Research on Cancer of the World Health Organization a decision was reached to establish a collaborative study aimed at testing the methodology of the measurement of dietary fiber in food. Nineteen laboratories took part in this work in Europe and in North America who were provided with aliquots of reference materials by the Dunn Nutrition Unit in Cambridge. D. A. T. SOUTHGATE (Food Research Institute, Norwich, England) described the protocol for the collaborative measurements. The participating laboratories were to use additional procedures they were familiar with in order to get a comparison between the results of the different analytical approaches. In December 1978, a meeting was held again in Cambridge to evaluate the results.

The book is a report on the afore-mentioned collaborative work containing the protocol for the measurements by SOUTHGATE, the reports of the contributors, the general discussion and a review of the different analytical methods and remaining problem by THEANDER, in altogether 16 chapters.

The reference materials were: wheat bran, rye flour A, rye biscuit A, rye flour B, rye biscuit B, apple pulp, citrus pectin, potato powder, soya flour.

The methods used by the participants fall into two main groups:

— methods based on direct carbohydrate determination,

— gravimetric methods.

The "unavailable carbohydrate" method, sometimes with slight modifications, was used by 11 laboratories: after fractioning, colorimetric determination of carbohydrates and GLC for measuring the monosaccharides were used. The composition of dietary fiber was given in terms of cellulose, noncellulosic polysaccharides and lignin.

Gravimetric methods, the Neutral Detergent Fiber method by VAN SOEST, and the enzymatic method of HELLENDOORN with some modifications were used by 8 participants. In two cases, the use of both methods was reported.

The amount of pectic substances was determined by the carbazole method in most cases. KATAN and VAN DE BOVENKAMP (Agricultural University of Dreijen, Wageningen) recommend a method based on $Cu^{2+}$ binding of polygalacturonic acid and THEANDER and ÅMAN (Swedish University of Agricultural Sciences, Uppsala) a decarboxylation method.

The results show great differences according to the method used for the determinations. The minimum and maximum values arrived at for the dietary fiber of standard samples are as follows (% fiber on dry matter basis):

| | minimum | maximum |
|---|---|---|
| wheat bran | 31.0 | 48.0 |
| rye flour A | 9.7 | 32.9 |
| rye biscuit A | 7.6 | 31.7 |
| rye flour B | 6.8 | 26.9 |
| rye biscuit B | 8.2 | 23.0 |
| apple pulp | 5.9 | 15.9 |
| citrus pectin | 0 | 99.9 |
| potato powder | 1.7 | 23.7 |
| soya flower | 2.6 | 19.6 |

The differences between the values obtained for the soluble and insoluble dietary fiber fractions by the different analytical methods were of similar extent.

These great deviations in the results of the different laboratories were mainly due to problems of removing the fat and starch content of the samples.

The results show that the analyst has to make a compromise according to his aim when choosing the method for determining dietary fiber and that further studies are needed first of all on sample preparation and pretreatment.

This most valuable study contains bibliographic references after each chapter. The methods are most suitable for application for further studies on the analysis of dietary fiber and the comparison of their applicability, reliability, accuracy etc. provide the possibilty of further developing idea.

Magda HORVÁTH-MOSONYI

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# XII. International Symposium on

# MICROBIAL ASSOCIATIONS AND INTERACTIONS IN FOODS

## 11–15 July 1983

Organized by

the Hungarian Society of Microbiology and
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under the auspices of the
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SCOPE OF THE SYMPOSIUM:

Types of microorganisms and their occurrence in foods as influenced by (a) intrinsic factors of foods, (b) extrinsic factors of storage and processing, and (c) implicit properties of microorganisms

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The scientific programme will comprise invited lectures, free reports, posters and discussions. Excursions to food factories and Lady's Programme will be organized. Language of the Symposium will be English.
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# CONTENTS

*Acta Alimentaria, Vol. 11 (4), pp. 327–341 (1982)*

# SENSORY OPTIMIZATION AND WAYS OF FINDING OPTIMAL COMBINATION

H. R. Moskowitz

(Received: 8 September 1980; revision received 15 June 1981; accepted: 17 July 1981)

This paper shows how it is possible to optimize consumer acceptance of food products. It provides the reader with three distinct types of optimization. The first concerns the optimization of ingredients. The second concerns the optimization of food acceptance, as a function of the serving frequency. The third concerns the optimization of combinations of foods, independent of serving frequency. From the sequence of optimization steps, the paper shows how it is possible to use optimization techniques at either the very simplest, psychophysical level (*vs.* ingredients), or at a complicated, higher order level of behavior (*i.e.*, combinations of food items).

This paper describes three studies, (a) psychophysical optimization of foods, (b) psychological optimization of components in a meal by varying interserving times and (c) psychological optimization of menus through evaluation of judgments of compatibilities. These studies exemplify three different approaches of applied psychology for the food industry. Each procedure is based upon a new use of well defined psychophysical and psychometric procedures. No special technology is needed to utilize each of these approaches in applied research. The difficulties which are encountered are primarily conceptual, rather than instrumental or analytical. Each method provides a different approach to optimizing consumer acceptance. The first does so by varying ingredients: a purely physical manipulation. The second does so by varying interserving time of foods – a little less "physical" and more psychological. The third optimizes psychological factors entirely.

## 1. Psychophysical optimization

In order to develop the most acceptable product, a manufacturer often must vary several interacting physical ingredients. Even the simplest foods, *e.g.*, an artificial fruit flavored beverage comprises a variety of components which interact with each other both physically and psychologically. For example an artificially flavored cherry beverage comprises sucrose, dextrose, cherry flavoring and acid.

In order to optimize product acceptability, the manufacturer must determine:

1. The relation between attribute sensory intensities (*i.e.*, sweetness, sourness, perceived flavor level) and physical concentrations of formula components.

2. Optima for the various components. Is there an optimum sweetener, optimum acid and an optimum flavoring level, when these are evaluated alone?

3. The effect of sensory attributes on one another. Does the perceived sourness mask or diminish the perceived sweetness, and vice versa? Is strength of perceived cherry flavor changed by mixtures of sucrose and acid, or is the perceived cherry flavor independent of the other components?

4. The effect of one ingredient on another. If sucrose is used, and the drink is left standing long enough will the sucrose be inverted by the action to a mixture of glucose and fructose (invert sugar) and made sweeter?

5. The direction and magnitude of change which is needed in a product to produce a more optimal product. Does the panelist possess an idea of where an optimum flavor intensity lies?

### *1.1. Relation between sensory intensity and physical concentrations*

Psychophysical scaling by magnitude estimation shows that for simple sensory continua (*e.g.*, sweetness, odor intensity, roughness, *etc.*), sensory intensity grows as a power function of physical intensity. The power function is written as: $S = kC^n$ (S = judged sensory intensity, C = physical intensity, such as sugar concentration, n = power function exponent, k = intercept). In these psychophysical experiments the panelist rates stimuli which vary on a stimulus continuum (*e.g.*, different concentrations of sucrose in solution, different concentrations of acid in a fruit punch). No constraints are placed on the numbers except that ratios of ratings should reflect ratios of sensory magnitude. For instance if the first sample is rated 15 and the second is rated 45, then we conclude that the second sample is perceived to be three times stronger than the first on the attribute chosen (*e.g.*, sweetness, sourness).

Figure 1 shows schematic curves for rated sensory intensity *vs.* physical magnitude. Some continua appears as accelerating curves (*e.g.*, the pain from electric shock applied to the fingers). Other functions appear as decelerating curves (*e.g.*, the brightness of light, the loudness of sound) (STEVENS, 1953). Some functions are straight lines (*e.g.*, perceived length). A logarithmic transformation of the stimulus intensities and the ratings often linearizes these curves. Those curves which are accelerating (concave upwards) have slopes greater than 1.0 (*e.g.*, the perceived intensity of shock in log-log coordinates). Those functions which initially appear decelerating have slopes less than 1.0. Those functions which were linear have a slope of 1.0.

The slopes of these functions are important for two reasons:

a) The slope of the function in log-log coordinates is the exponent (N) of the power function. Thus the exponent for shock is greater than 1.0. The exponent for brightness is less than 1.0.

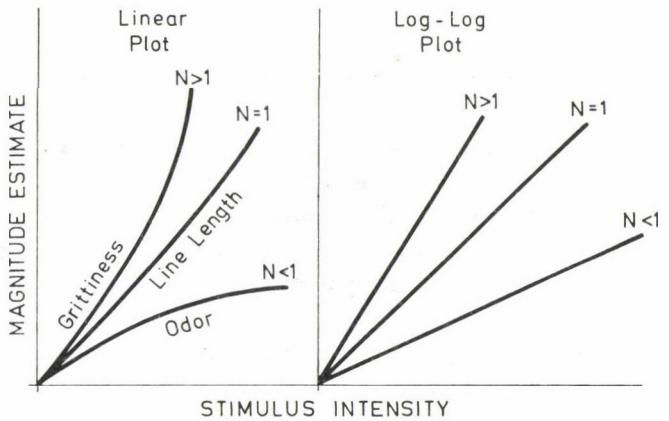


Fig. 1. Schematic functions for sensory intensity obtained by the method of magnitude estimation. The functions when presented in linear form are usually curved, but the logarithmic transformation linearizes them. This linearization implies that the sensory – instrumental relations can be well represented by power functions, which become straight lines in log-log coordinates. N = Power Function Exponent

b) The exponent of the power function reveals how we respond to stimulus changes. If the exponent exceeds 1.0, then the panelist *expands* the range of the physical intensities to a larger perceptual range. Assume that the exponent $n$ is 2.0. Then, a 10-fold range of intensities becomes a subjective range of $(10)^2$ or a 100-fold range of sensory intensities. If the exponent is 1.5, then the same 10:1 range becomes approximately a 32:1 subjective range. Doubling the stimulus for a continuum governed by exponent 2.0 produces a 4-fold increase. A contraction occurs for exponents lower than 1.0. A 10-fold increment appears as a smaller increment to the observer. If the exponent is 0.5, then the same 10:1 range becomes approximately a 32:1 subjective range. Doubling the stimulus for a continuum governed by exponent 2.0 produces a 4-fold increase. A contraction occurs for exponents lower than 1.0. A 10-fold increment appears as a smaller increment to the observer. If the exponent is 0.5, then the 10-fold increase appears only as an increment of 3.2 ($10^{0.5} = 3.2$).

If it were possible to measure the entire physical range of a chemical in a food (*e.g.*, the amount of sour citric acid added to a fruit punch) then one could characterize the entire range of sourness by a single number, corresponding to the total psychological range of sourness. This range is the ratio of the

highest to the lowest concentration (H/L) raised to the exponent of the sourness function (*e.g.*, 0.7; MOSKOWITZ, 1971). *E.g.*, for the food being studied, the psychological range is $(H/L)^{0.7}$. When the psychophysical function *is not* a simple power function other methods of summing the component sensory intensities should be applied. These are available from the methods of calculus.

### *1.2. Characterizing mixture rules according to psychophysics*

Foods are chemical mixtures, whose exact components are often poorly understood. Many physical and chemical interactions occur. There is usually interaction among the sensory attributes as well. Various studies suggest a wide range of such psychological interactions. We consider the example of taste mixtures in detail to exemplify the psychophysical approach to mixtures of food components.

*Taste Mixtures:* Adding sucrose to coffee reduces the bitter taste (*e.g.*, the caffeine taste, among others). The sweetness of the sucrose is also reduced. As a general rule, the addition of unlike-tasting materials to each other produces mutual suppression. Each component reduces the taste intensity of the other.

The history of research in taste mixtures is long, complicated, and full of disputes about particulars. A quick overview is necessary so that the food technologist can appreciate the complete sensory effects which occur, and which can affect taste. Taste suppression is not symmetric – sucrose added to caffeine or to quinine sulfate suppresses bitterness differently than bitterness suppresses sweetness. It is difficult, *a priori*, to predict the extent of suppression for each taste in the mixture. BEEBE–CENTER and co-workers (1959) measured the taste intensity of mixtures of sucrose and sodium chloride. Taste suppression increased with the level of masking agent. As added sodium chloride continually increased, mixture sweetness continually diminished. MOSKOWITZ (1972b) suggested a general rule based upon both the BEEBE–CENTER and co-workers (1959) study, and on studies of the taste of glucose or fructose mixed with NaCl, citric acid and quinine sulfate. The *total* amount of suppression is approximately the same (*viz.* 40–60% of the starting intensity). If the experimenter knows the perceived taste intensities of the two components in the mixture, then the *total* mixture taste intensity will be about half as strong as would have been predicted on the basis of the arithmetic sum of the taste intensities of the two components evaluated separately. That 50% is *not necessarily distributive*. The sweetness is not reduced by 50%, nor is the saltiness (or sourness or bitterness) also reduced by 50%. Rather, the *sum* is reduced to half of what it would normally be. One taste may predominate in the mixture and the other taste may be entirely suppressed.

In mixtures of tastes which have different qualities, mixture taste quality may change. The sweet quality in the presence of acid may differ from the sweet quality of sucrose evaluated alone in aqueous solution. This quality shift is particularly important for the product developer who must use sweetener in the presence of acid. More than 50 years ago HAMBLOCH and PUSCHEL (1928) reported that a mixture taste (Mischgeschmack) emerged when NaCl and sucrose were tasted together in water. MOSKOWITZ (1972b) replicated this effect which he labelled as "clashing". Clashing was akin to the perceptual clashing of two incompatible stripes alternating with each other (*e.g.*, green and red stripes).

If the product developer mixes odorants (flavors) of different types rather than taste materials, new aromas may emerge. Unlike taste mixtures (which seem to be analyzable to component sweet, salty, sour, and bitter), odor mixtures *may* produce a homogeneous new impression. The quality may be similar to the component qualities, but retain its own emergent identity. As yet little information exists to predict the qualitative effects when two flavors are mixed, except from experience with a variety of flavor mixtures. Quality creation, is still the "art of the perfumer and flavorist" not the domain of the psychophysicist. The product developer often mixes together two taste or aroma components which have the same or similar quality. Usually, components of similar quality add together. The result is a perception of greater intensity. Studies of taste mixtures with sweeteners or acids reveal that the components add in an almost arithmetic fashion. This summation, and a small correction factor (determined through experimentation) allows the product developer to predict mixture taste intensity, and to vary the component concentrations to:

a) Maximize sweetness or sourness subject to a fixed cost.

b) Minimize cost subject to a fixed mixture sweetness (MOSKOWITZ & WEHRLY, 1972).

### *1.3. Psychophysical optimization through direct judgments of desired change*

The panelist can evaluate foods using a liking scale, to measure degree of liking and disliking. With a set of products varying along a single dimension (*e.g.*, sucrose in a beverage) the ratings generate a preference-concentration function. In the usual back-and-forth test procedures the product developer searches for that formula level which produces the highest preference rating. Further attempts at refining the optimum level are made when the product developer feels that no further refinements are either necessary or cost/effective.

The foregoing procedure permits the panelist to act as a judge but not actively indicate the desired amount of change. It seems reasonable that panelists do possess a concept of the "ideal product" albeit, perhaps a nebulous

one. When evaluating products the panelist may compare this ideal product to the product being presented, and rate the current product against the ideal. It would be useful to determine what the panelist uses as his or her ideal (*e.g.*, in terms of its attributes).

The use of the panelist's ideal product seems to be a reasonable concept if we consider how an individual prepares food (*e.g.*, a soup). During the cooking process one often samples to determine whether sufficient salt or seasonings have been added, or often whether the "optimum" has been exceeded. The cook knows approximately the amount of salt or seasonings which have to be added. Although the precise amount may require several tries the cook at least knows the general direction of change, and the approximate magnitude of such change.

This approach using the self-designed ideal product lends itself to psychophysical procedures for relatively simple physical food systems, such as soups, gravies, and beverages. It also lends itself to such relatively non-interactive aspects of food such as texture and color (when artificial coloring is added to a food in order to improve its appearance).

MOSKOWITZ (1972a) elaborated a product optimization approach, in which the panelist actively participates in testing. In that study the panelist judged the direction and amount in which the food should be modified, in order to be acceptable.

MOSKOWITZ's (1972a) study provided two different approaches to optimize, and measured product acceptability to validate the optimization. The studies followed this sequence:

1. The panelist evaluated samples which varied along a single physical attribute, or on a limited number of attributes. In one study the panelists evaluated hamburgers with varying grind size. The hamburger meat was chopped with a machine which produced different sizes. The second study investigated the acceptance of varying mayonnaise levels in a tunafish spread. The third study investigated the variation in both the sugar (sucrose content) and flavor level (cherry + citric acid) of a cherry-flavored beverage.

2. The panelist rated perceived intensity of each attribute, using either magnitude estimation or a category scale. The scaling related perceived sensory intensity of the attribute to the physical level. For magnitude estimates of perceived chunkiness, a power equation $S = kC^n$ ($n$ around 0.5), showed that panelists reduce the range of physical grind variation to a much smaller range of perceived grind. For perceived sweetness and cherry flavor of beverages *vs.* cherry level and sucrose power functions had exponents around 1.1–1.4. The perceived amount of mayonnaise in tunafish spread scaled by magnitude estimation showed an exponent of 1.2. The perceived amount of mayonnaise in tunafish spread using category scaling was described by a logarithmic function.

3. The panelist then estimated the change by which he or she would like to increase or decrease the sensory level of mayonnaise for each sample in order to reach the optimum. This estimation was done concurrently with the evaluation of the perceived mayonnaise level in each sample.

4. Except for informal instruction in scaling, panelists had no initial familiarization with optimum samples, nor with the expected flavor and texture characteristics. The panelists participated as ordinary consumers (that is, an "untrained" or "non-expert" panel). For the experiment it was assumed that the panelist had a concept of what an ideal product level would be for each attribute (grind size in hamburger, cherry and sweetness level for beverage, mayonnaise level for tunafish spread).

5. The *desired amount of change* for each level was used in conjunction with the then-current level of the product attribute to estimate a new level, which would be closer to the ideal. For example, in the magnitude estimation evaluation, the panelist estimated size of grind for sample $X$, as well as a ratio of desired change to improve the food (*e.g.*, $2X$, $0.33X$). The product of these two numbers (original value $\times$ amount of change) generated a new sensory value corresponding to the optimum. Each product itself produced an independent estimate of the optimum. For category scaling the procedure was similar. The panelist estimated the number of categories he would like to add or subtract from the current rating. The optimum was computed as Current Sensory Level + Number of Categories Changed.

6. It was straightforward to determine that level of the ingredient which corresponded to the desired optimum psychological level, by interpolation.

7. In order to validate the optimization panelists rated their liking of each product formulation. They did the acceptance test at another session. The most acceptable sample was also the one which lay closest to the optimum. Often the optimum level lay intermediate between two samples. The acceptance testing of the test products did not include the level selected to be the optimum. In an optimization procedure a second phase would include those intermediate levels.

8. Other tests and analyses were also performed in the context of the optimization procedure. For example, in the study of cherry flavored beverages the panelists estimated both the level of sweetness and the level of cherry flavor, as well as the optimal level of each. This generates a two dimensional array of samples. Both sucrose concentration and beverage flavor level varied. The set of combinations was reduced to a set of possible optimal levels, by the first set of evaluations. A second pass was made with the reduced set of feasible concentrations of sucrose and beverage flavor level, to reach a single optimal concentration.

9. The development of optimal concentrations was considerably shortened by two modifications. In one, the panelist indicated that sensory level at

which he thought the optimum lay, after rating each of the samples. This approach yielded on optimum level, derived from the scaling of perceived sensory magnitude, rather than several optima, each corresponding to separate stimuli. The panelist integrated the entire array of perceptions of the product, to make the rating. Through his own knowledge of his scale for magnitude the panelist then assigned a single number to reflect the optimum. This *one step approach* produced optima similar to the multistep approach, which utilized responses to each stimulus.

In the second modification, the stated degree of change was plotted as a function of the concentration of the flavor ingredients, in order to determine where the *cross-over point* occurred between increase and decrease. Linear functions often fitted these relations. There were only small differences between optima obtained by this procedure and optima obtained by the multi-step procedure.

In conclusion, for relatively simple stimuli, psychophysical optimization is within the grasp of the food product developer. Interactions among ingredients, however, may necessitate more complicated stimuli to test, if the interactions produce modifications of sensory magnitudes. Whether the panelist rates a taste, a flavor or a texture attribute, seems to make little difference – the procedure appears to work as well with taste (sucrose sweetness) as with flavor (cherry level). Even complicated stimuli, such as the perceived level of mayonnaise in a food, are amenable to be optimization procedure as well.

## Optimization of psychological variables

Psychological analysis of food concerns how the panelist perceives relations among subjective variables. A simple example will clarify the distinction between psychophysical and psychological analyses. In the study of force, the psycho-physicist studies the relation between subjectively perceived force ($F_s$) and physically measurable force (F). The psychological analyst studies how the observer combines perceived mass ($M_s$) and perceived acceleration ($A_s$) to yield an impression of perceived force. Does the observer respond to the product (MA), and if so, is the psychological equation the following?

$$F_s = (M_s)\,(A_s)$$

In order to optimize or maximize the psychological function, the investigator should follow this strategy:

(a) Scale the appropriate psychological variables.

(b) Determine how these variables combine. Do the variables add, multiply, *etc.* What is the nature of the combination operation which joins together the different psychological variables.

(c) Develop an equation which represents the dependent psychological variable one wants to optimize as a function of component and independent psychological variables.

(d) Optimize or maximize the dependent psychological variable by varying the independent components.

One illustration of this approach is menu planning. Menu planners must develop meals which are nutritious, attractive, and relatively inexpensive. The information that they use to develop the meal includes the nutritional values for each food item in the menu inventory (*i.e.*, vitamins, minerals, calories), the cost/per food serving, the preferences for the items, *etc.* BALINTFY and co-workers (1972), and ECKSTEIN (1967) discussed methods to optimize menus. The reader should keep in mind that we are optimizing menu acceptance by optimizing the selection of menu components.

A study on military menu optimization illustrates the steps in menu optimization. The study problem was to develop a *menu index*, which would provide a single number to indicate the acceptability of a menu, and then maximize that index value. The menu items comprised foods consumed by the military services of the U.S. Government.

In order to develop a menu index, the menu was divided into five item classes: Entree, Vegetable, Starch, Salad and Dessert. A general expression was developed to relate the menu preference of soldiers to three characteristics of the foods:

1. Inherent or basic preference of soldiers for the item, P.
2. The change in preference as a function of the inter-serving interval. Foods served too frequently lose their acceptability, sometimes quickly, and sometimes only after many repeated servings. This is the time factor, T.
3. A weight for "importance" that soldiers place on the different items in the meal. The soldier assigns higher weights to certain menu classes (*e.g.*, entrees) than to other classes (*e.g.*, vegetables). This is the importance factor, I.

Given these three characteristics of each food item, one can then develop an equation to combine the characteristics and predict acceptance. How P, T, and I combine does not necessarily have an immediate relation to the *values* of P, T, and I. The following additive equation was chosen because of its conceptual and quantitative simplicity:

$$\text{Menu Preference} = (\text{PTI})_e + (\text{PTI})_v + (\text{PTI})_{st} + (\text{PTI})_{sa} + (\text{PTI})_d + \text{constant}$$

(e = entree, v = vegetable, st = starch, sa = salad, d = dessert)

The equation says that preference or acceptance of a menu is the product of the items preference acceptance value (P), the weighting factor for the

importance of the item in the array of items (I), and the time factor (T). These three are multiplied out for a food and then summed over all items in that menu. The menu index expression is *arbitrary*. It was chosen for quantitative simplicity. It assumes *no* interactions among the different menu items.

In order to estimate P, T, and I values for foods, we used a two-part questionnaire. The soldiers first rated how much they would like each of 140 food items (from the five menu classes), under the assumption that the last time that they had eaten the food was 3 months previous, 1 month, 2 weeks, 1 week, 3 days and the day before. These ratings in part A of the questionnaire generated acceptance (or preference) values as a function of time since last serving. Respondents used the standard 9 Point Hedonic Scale of Food Preference (PERYAM & PILGRIM, 1957). The experimenter could estimate the preference for the item when evaluated alone (at various time intervals).

Part B of the questionnaire presented the soldier with 136 different menus. Each menu contained 1 entree, 1 vegetable, 1 starch, 1 salad and 1 dessert. Each item in Part B had been previously surveyed in Part A. For each menu in Part B the respondent assigned a Hedonic Scale value to indicate his degree of liking and disliking of the *entire menu*.

The two parts of the survey provided sufficient information for the following analyses:

a) For each of the 140 items surveyed alone (Part A) the following equation was developed: $P = k_1 + k_2 (\log T) + k_3 (\log T)^2$. The equation relates preference for a single item ($P$) to the time in days since it was last served ($T$). The logarithmic equation is often used to relate category scale responses ($P$), to ratio scale values which serve as the independent variables ($T$). The logarithmic value of $T$ was augmented by a quadratic term $(\log T)^2$ since the preference function often flattened after month 1. For large time intervals the change in preference was not nearly as dramatic as the change in preference for shorter time intervals.

b) For each respondent, the preference values for every one of the items surveyed was related to the preference value assigned to the entire menu. Since there were 136 such menus, the following equation was developed for each respondent:

$$\text{Menu Preference (Menu } i) = K_1 P_e + K_2 P_v + K_3 P_{st} + K_4 P_{sa} + K_5 P_d + K_0.$$

The dependent variable was menu preference. The independent variables were the component preferences (at 3 months). The matrix of 136 sets of observations or "cases" (corresponding to the 136 different menus) was analyzed by least-squares regressions, separately for each of the 175 respondents participating in the survey and for the average across respondents. The weights ($K_1 \ldots K_5$) attached to each component preference illustrated the importance value of each menu class.

c) The solution of the 175 least squares functions, one per respondent, produced one set of weighting factors for each respondent. There was a unique weight of the menu items in a meal of 5 components for each individual. Some individuals highly weighted starches, other highly weighted the entrees, etc. The respondents concurred about the importance of starches and vegetables, but differed quite widely in the emphasis upon the entrees. Several respondents rated the entire menu principally by how much they like the entree, and ignored the remaining items in the menu.

The general weighting function was expressed by the following two equations. The first contains an additive constant and the second has no additive constant:

$$P = 2.07\,P_e + 0.53\,P_{st} + 0.42\,P_v + 0.25\,P_{sa} + 0.57\,P_d + 5.68$$

$$P = 2.34\,P_e + 0.74\,P_{st} + 0.58\,P_v + 0.35\,P_{sa} + 0.76\,P_d + 0$$

(no additive constant used).

d) A menu index was constructed. It accounted for the inherent preference of items (D), the time factor which diminished preference (T), and the importance of the items in the meal (I). Each menu obtained a single index number. With the index number the menu planner could evaluate the changes in preference for meals later in the menu cycle if a change was made at the present time.

A key advantage of the model is the possible optimization of menu acceptance. A variety of approaches can be used (BALINTFY *et al.*, 1975).

One approach to optimizing is to develop a menu which is preferred "maximally" (*viz.* a preference menu), without any consideration of cost. To do this requires a knowledge of the menu index, and a mathematical way of optimizing that index. Two ways were chosen, (a) the "brute-force" computer method of scanning each day for the most preferred items, and (b) the "analytical approach" *via Lagrangian* multipliers, and the techniques of differential calculus.

In the "brute-force" method the items were available each day for selection. The computer was instructed to choose the most acceptable entree, the most acceptable vegetable, *etc.*, which generated five component menu, each item of which was maximally acceptable for the group at that time. Afterwards, in preparation for the next day, these five items were then downgraded in their acceptability, according to their respective time-preference formula. Items which had not been chosen were upgraded in their preference, again by their appropriate time-preference formulas.

Each time an item was chosen the preference formula was recycled to $T = 1$ (since it was chosen the previous day), whereas the items that were not chosen increased their preference from $P = k_1 + k_2 (\log T) + k_3 (\log T)^2$ up

to $k_1 + k_2 [\log(T + 1)] + k_3 [\log(T + 1)]^2$. This process was repeated for each day of the 42 day menu cycle, and then the entire cycle was repeated a dozen times until a steady-state was reached. The initial choice of items, the initializing times since last serving, *etc.*, influences the first optimum menu cycle. A *Monte-Carlo* procedure, and a dozen or so runs through the cycle served to equalize all items in terms of starting bias. The menus thus chosen were 'optimum' in terms of the fact that no other arrangement of items could yield a more acceptable menu index for that day.

In the *Lagrangian* approach the optimum inter-service interval was computed from the time-preference functions. These intervals were then used to select foods for menu cycle. The results agreed quite closely with those obtained by the "brute-force" technique. In both situations some modifications in the time-preference functions eventually had to be made in order to avoid some conditions of non-optimality, and finally a modified time-preference function was used which incorporated a memory factor as well.

## Optimizing compatibilities of foods in menus

Some foods are compatible with just a few other menu items. Other foods are compatible with many menu items. For instance, ham and sweet potatoes is a traditional American combination. Sauerkraut and turkey is not a traditionally compatible combination of entree and vegetable, although frankfurters and sauerkraut are highly compatible.

In order to optimize compatibilities, another approach was required. This procedure used the principles of analytic geometry and multidimensional scaling to optimize purely psychological characteristics of a menu. The procedure and the results are summarized as follows:

a) Respondents rated the compatibility of pairs or triples of food items (5 entrees, 5 vegetables, 5 starches). The panelists were instructed to disregard how much they liked each of the items in the combination, separately (MOSKOWITZ *et al.*, 1977).

b) This survey produced a matrix of compatibilities. The compatibilities were interpreted as *inverses* of inter-food distances. Those foods which were rated high on compatibility were separated by small inter-food distances in the geometrical space. Incompatible food pairs were separated by much larger distances in the same space. The foods thus become points in a food geometry.

(c) The questionnaire yielded a $15 \times 5$ matrix of foods. Parts of the matrix were empty (*viz.* inter-food distances or compatibility ratings between pairs of entrees, vegetables, potatoes, respectively, which were never directly evaluated in the questionnaire).

d) A computer program MDSCAL 5M (KRUSKAL & CARMONE, 1969) located the 15 foods in a 3 dimensional space. The multidimensional scaling

approach juggled the position of the points (*viz.* foods) in the three dimensional space until the rank order of inter-food distances in the space most highly correlated with the inverse of the judgements of compatibility. This approach (called non-metric multidimensional scaling) located all 15 foods in the three dimensional space. Figure 2 shows the first two dimensions of the space.

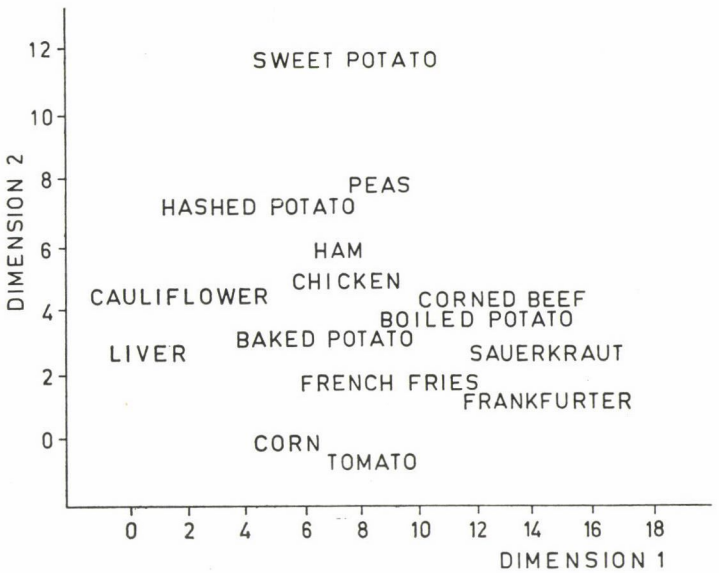


Fig. 2. Two dimensional compatibilities map of 5 entrees, 5 starches, and 5 vegetables, obtained from direct magnitude estimates of pairwise item compatibilities, and reanalysis of these judgments by MDSCAL 5M

e) The geometrical space contained all of the foods. The geometrical coordinates of each food in the space allowed the computation of (a) the Euclidean distance between any pair of foods, and (b) the center of gravity (centroid) for any set of 2 or more foods.

f) Another computer program generated *aggregate* and *segregate* menus, which showed optimal product compatibility. The computer program selected an entree (either at random, or else a pre-designated one). It then followed a specific *search strategy* to find a food compatible with *that* entree. The computer found the closest vegetable to that entree, then the closest starch (relative to the entree, or else relative to the center of gravity of the entree/vegetable cluster). The search continued until a set of foods had been selected which all lay near each other. Each entree was fairly interchangeable with every other entree in the cluster in terms of compatibility with starches and vegetables. This strategy produced an *aggregate menu.* All items are clustered together. Each entree is compatible with each starch. Items in the same menu

Table 1

*Sample menus generated from compatibilities*

Type I: Aggregate menus

Choice of single items from each of three menu classes:

1) Roast Chicken or Baked Ham, Buttered Cauliflower or Buttered Peas, Hashed Brown Potatoes or Boiled Potatoes.
2) Roast Chicken or Grilled Liver, Buttered Cauliflower or Buttered Peas, Hashed Brown Potatoes or Boiled Potatoes.
3) Corned Beef or Frankfurters, Buttered Peas of Sauerkraut, Boiled Potatoes or Baked Potato.

Type II: Segregate menus

Choose one menu or another:

1) Baked Ham, Buttered Peas, Boiled Potatoes or Frankfurters, Sauerkraut, Baked Potato.
2) Baked Ham, Buttered Peas, Boiled Potatoes or Grilled Liver, Creamed Corn, Hashed Brown Potatoes.
3) Corned Beef, Buttered Peas, Boiled Potatoes or Grilled Liver, Cauliflower, French Fries.
4) Corned Beef, Buttered Peas, Boiled Potatoes or Grilled Liver, Creamed Corn, Hashed Brown Potatoes.
5) Chicken, Buttered Cauliflower, Hashed Brown Potatoes or Frankfurters, Sauerkraut, Baked Potato.

class can be interchanged. The aggregate menu is the preferred menu if the panelist has many food choices that are essentially interchangeable. *Segregate* menus were also obtained by the computer. Segregate menus are combinations of food items which *go well with each other*, but in general none goes well with other items. In the geometrical food space the segregate menus are isolated clusters, lying far away from other clusters. In contrast, aggregate menus appear as large amorphous clusters of items. Table 1 compares the aggregate and segregate menus.

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Address of the author:

Dr. HOWARD R. MOSKOWITZ* Weston Group, Inc.
60 Wilton Road
Westport, CT 06880
USA

* Present address: Moskowitz/Jacobs Inc.
111 North Central Ave
Suite 390
Hartsdale
New York 10530
USA

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# STUDIES ON *ASPERGILLUS FLAVUS*

## III. CHEMICAL SENSITIZATION OF *ASPERGILLUS FLAVUS* SPORES TO THERMORADIATION

S. R. PADWAL-DESAI, A. S. GHANEKAR and ARUN SHARMA

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The thermoradiation treatment was found to be more effective in bringing down the survival of *Aspergillus flavus* spores as compared to irradiation alone, at ambient temperature. The spores of the non-toxigenic strain showed higher sensitivity to both irradiation as well as thermoradiation treatments. Heat followed by irradiation caused greater destruction of spores as compared to the radiation followed by heat. Among the three sensitizers used in the studies, the maximal sensitization was observed with N-ethylmaleimide, to both irradiation as well as thermoradiation treatment. The irradiated and thermoradiated spores showed significant recovery upon holding which was not observed with the spores given similar treatment after sensitization.

Several chemical agents are known to enhance the sensitivity of the living cell to irradiation when present in the system during treatment (SOMMER *et al.*, 1971). The spores of fungi have been shown to be sensitive to radiations and the sensitivity could be enhanced by chemicals and heat (SOMMER *et al.*, 1971; PADWAL-DESAI *et al.*, 1976a, b). The spores of toxigenic and non-toxigenic strains of *Aspergillus flavus-parasiticus* group are ubiquitous in nature and the toxigenic strains of this group are known to elaborate hepatotoxic secondary metabolites, aflatoxins, whenever the conditions are conducive for their growth (MAGGON *et al.*, 1977). Thermoradiation has been shown to have practical relevance to the destruction of the microbial spores in food materials (FISHER & PFLUG, 1977; PADWAL-DESAI *et al.*, 1976a, b; 1979). In the present studies chemical sensitization of *A. flavus* spores to thermoradiation was investigated using iodoacetamide, N-ethylmaleimide and vitamin $K_5$, which are well known sensitizers of microbial systems.

### 1. Materials and methods

*Organism*: The toxigenic strain of *Aspergillus flavus* was isolated from peanuts, whereas the non-toxigenic strain of *A. flavus* was isolated from bread (PADWAL-DESAI *et al.*, 1976a). The aflatoxin producing ability was confirmed by growing the isolate in a synthetic medium, extraction of the culture broth with chloroform and subsequently running a thin-layer chromatography along with the authentic samples of aflatoxins (JONES, 1972).

*Chemicals*: Iodoacetamide (IAM) was purchased from Sigma Chemicals. N-ethylmaleimide (NEM) was the product of M/a. Fluka Switzerland, whereas vitamin $K_5$ (vit. $K_5$) was procured from Calbiochem (USA).

*Preparation of spore suspension*: Harvesting of the spores of *A. flavus* and the preparation of spore suspension was carried out according to the method of PADWAL-DESAI and co-workers (1976a).

*Sensitization studies*: The stock solution of the three sensitizers were prepared in prechilled distilled water and sterilized by *Seitz* filter. During the experiments one ml aliquot from either the stock solution or its dilution (S) was added to 100 $cm^3$ spore suspension (ca $10^6$ spores/$cm^3$) so as to have the desired level of the sensitizer. The spore suspension was allowed to incubate on a rotary shaker for 12 h at 0.4 °C. The aliquots from the incubated suspension were then subjected to irradiation or thermoradiation treatment. The aliquots of the spore suspension given above treatments were also similarly incubated, but without the presence of sensitizer to serve as control. Thermoradiation treatment was given in two ways. In one set of aliquots of spore suspension, heat (50 °C $\pm$ 0.5/5 min) was applied first followed by irradiation, whereas, in another set, irradiation was followed by heat. Irradiation was carried out in a *Gamma Cell* 220 (Atomic Energy of Canada Ltd., 22 500 Ci; 1.1 kGy $h^{-1}$). The procedure for irradiation, heating and the combined thermoradiation treatment have been described earlier (PADWAL-DESAI *et al.*, 1976a). The surviving fraction was determined by serial dilution and plating on potato-dextrose agar using the standard pour plate technique and the plates were incubated at 25 °C $\pm$ 1 for 72 h.

*Recovery studies*: An aliquot from the sensitized and the unsensitized spore suspension described above was diluted 100 $\times$ in distilled water and was maintained on a rotary shaker at 25 °C for 96 h and the spore count was determined every 24 h by the standard pour plate technique as outlined above. The percent survival either against dose of irradiation or time of incubation was plotted on the semilog paper with each point consisting of an average of three readings.

## 2. Results and discussion

It was observed that below $2.5 \times 10^{-3}$ *M* level, the sensitizers used in this study were non-lethal to the spores. Also a 12 h incubation of the spores with the sensitizers prior to treatment was enough to cause maximal sensitization. Hence the spores of the two fungi were exposed to non-lethal concentrations in the range of $2.5 \times 10^{-4}$ *M* to $2.5 \times 10^{-5}$ *M*, of each of the three sensitizers, for a period of 12 h. Such a long incubation of spores with sensitizers is needed only in case of the spores having low metabolic activity and thus a very slow solute uptake. However, yeast cells, which are in a high state of metabolic

activity do not require such a long incubation (DUPUY & TREMEAU, 1966). Figure 1 shows the effect of two concentrations of IAM on the sensitivity of the spores to gamma irradiation. As compared to the control, the surviving fraction of the cells pre-incubated with the sensitizer was reduced considerably.

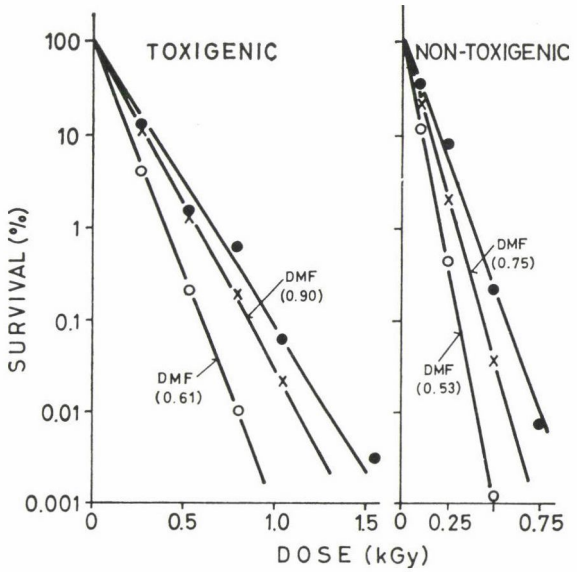


Fig. 1. The effect of IAM on radiation survival of *Aspergillus flavus* spores. A portion of the spore suspension was incubated with the different concentrations of IAM for 12 h at 0–4 °C on a rotary shaker. The aliquots from the incubated suspension were then subjected to the irradiation and combination treatments. The surviving fraction was determined by the standard plate count method (SPC). In case of the controls the suspensions were not treated with the sensitizers. All plotted values are the averages of 6 replicates from two experiments

—●— control
—×— $2.5 \times 10^{-5}$ *M* IAM
—○— $2.5 \times 10^{-4}$ *M* IAM

The increased concentration of the sensitizer also caused progressive destruction. It is significant that the spores of the non-toxigenic strain were sensitized to a greater extent than the spores of the toxigenic strain. The comparison of the values of dose modification factor for the curves with the different concentrations of the sensitizer would also confirm this observation. Dose modification factor (DMF) has been defined as the ability of the sensitizer to enhance the radiation induced lethality of the fungal spores, as represented by the ratio of the doses of radiation required for the same extent of survival in the presence and absence of the sensitizer.

Figure 2 depicts the sensitization of the spores of the two strains by vit. $K_5$ and NEM. The DMF values with NEM show maximal sensitization effect as compared to that with vit. $K_5$ and IAM (Fig. 1). Once again the spores

from non-toxigenic strains showed more susceptibility to the sensitizer. This could perhaps be attributed to the possible biochemical and/or morphological differences between the spores of the two strains.

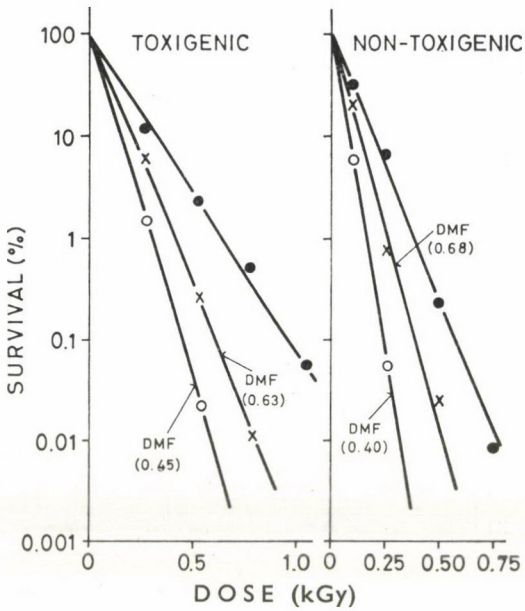


Fig. 2. The effect of vit. $K_5$ and NEM on radiation survival of *A. flavus* spores. A portion of the spore suspension was incubated with the sensitizer for 12 h at 0–4 °C on a rotary shaker. The aliquots from the incubated suspensions were subjected to treatments. The survival was determined by SPC method. The suspension not incubated with the sensitizer was used as control. All plotted values are the averages of 6 replicates from two experiments.

—●— control
—×— $2.5 \times 10^{-4}$ *M* vit. $K_5$
—○— $2.5 \times 10^{-4}$ *M* NEM

The sensitization of the spores, given thermoradiation treatment, by the three sensitizers has been depicted in Fig. 3. As the non-toxigenic spores were found to be more sensitive they were subsequently given half the radiation dose given to the spores from toxigenic strain. As observed from the Fig. 3, irradiation followed by heat was less effective as compared to the heat followed by irradiation, in bringing down the survival, and consequently more sensitization was observed in the latter. Maximal sensitization was seen with NEM, followed by vit. $K_5$ and IAM as indicated above. The combined treatment with heat and radiation was more lethal as compared to irradiation alone. The thermal treatment at 50 °C/5 min seems to be innocuous and non lethal. It is nevertheless obvious that prior heat treatment helped irradiation to be more effective.

Figure 4 depicts the recovery profile of irradiated, irradiated sensitized, thermoradiated and thermoradiated sensitized spores of toxigenic *A. flavus*. It was observed that IAM-sensitized conidia of *A. flavus* to irradiation, do not

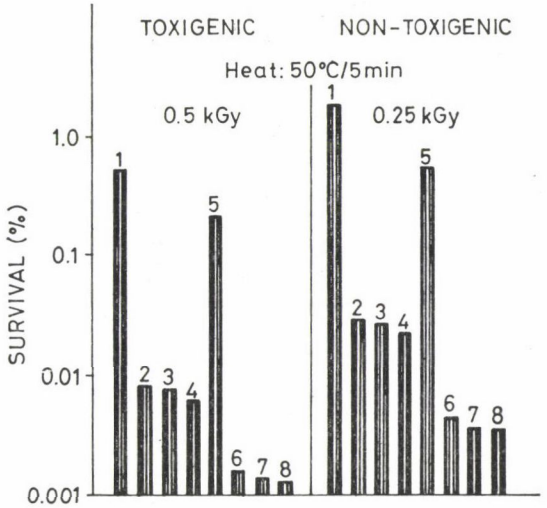


Fig. 3. The effect of thermoradiation sequence and chemical sensitization of *Aspergillu flavus* spores to thermoradiation treatments. 1: Irradiation + Heat, 2: Irradiation + Heat (IAM treated), 3: Irradiation + Heat (vit. $K_5$ treated), 4: Irradiation + Heat (NEM treated), 5: Heat + Irradiation, 6: Heat + Irradiation (IAM treated), 7: Heat + Irradiation (vit. $K_5$ treated), 8: Heat + Irradiation (NEM treated)

show recovery. As a result, with optimum non-toxic concentration of IAM, more lethality is apparent while conidia treated with 0.5 kGy alone or in combination with post-heating at 50 °C showed about 10-fold recovery after 96 h, the same was insignificant in pre-heated irradiated conidia up to 72 h. After 96 h, however, slight recovery was evident. Results of the IAM soaked conidia with pre- or post-heat treatments to 0.5 kGy irradiation show total inactivation even at 24 h of holding period. Results on the recovery studies of NEM and vit. $K_5$ treated conidia of both the fungi after heat, irradiation and combination treatments are summarised in Table 1. It is seen from the Table that NEM and vit. $K_5$ have affected the recovery process of both the organisms, when treated with radiation. No further lethality, however, is apparent, as was observed for IAM treated conidia of *A. flavus* (Fig. 4). Further, a total inactivation is observed in combination treated conidia during the holding period. The mechanism of heat and radiation synergism has already been explained by FISHER and PFLUG (1977), and PADWAL-DESAI and co-workers (1979). According to FISHER and PFLUG (1977), the synergistic effect is a consequence of the need to degrade a single or a pair of vital macromolecules at multiple locations in order to "kill" the microbial spore.

Table 1

*Effect of NEM and vit. $K_5$ ($2.5 \times 10^{-4}$ M conc.) on recovery of conidia of* A. flavus

| Organism | Sensitizer | Survival (%) | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | 0 h | | | 48 h | | | 96 h | | |
| | | Irr. | Irr. + Heat | Heat + Irr. | Irr. | Irr. + Heat | Heat + Irr. | Irr. | Irr. + Heat | Heat + Irr. |
| *A. flavus* | NEM | 0.023 | 0.0062 | 0.0014 | 0.024 | 0.0060 | 0.0013 | 0.030 | 0.0059 | 0.0010 |
| (toxigenic) | Vit. $K_5$ | 0.26 | 0.0076 | 0.0015 | 0.28 | 0.0073 | 0.0014 | 0.29 | 0.0071 | 0.0010 |
| *A. flavus oryzae* | NEM | 0.056 | 0.023 | 0.0035 | 0.057 | 0.022 | 0.0033 | 0.061 | 0.020 | 0.0030 |
| (non-toxigenic) | Vit. $K_5$ | 0.75 | 0.027 | 0.0036 | 0.76 | 0.026 | 0.0033 | 0.81 | 0.022 | 0.0029 |

The conidia of toxigenic and non-toxigenic strains of *Aspergillus* were soaked in sensitizers for 12 h and were irradiated at 0.5 kGy and 0.25 kGy doses, respectively with pre- and post-heating at 50 °C for 5 min. The % survival from 0–4 days was determined as described in text. Both chemicals affected the ability of irradiated conidia to recover, while in combination treated conidia, no recovery was observed.

PADWAL-DESAI and co-workers (1979) have observed that besides direct DNA damage thermal sensitivity of various enzymes participating in the repair process might be the underlying cause for the increased susceptibility to thermoradiation. That the repair processes are quite active in irradiated and ther-

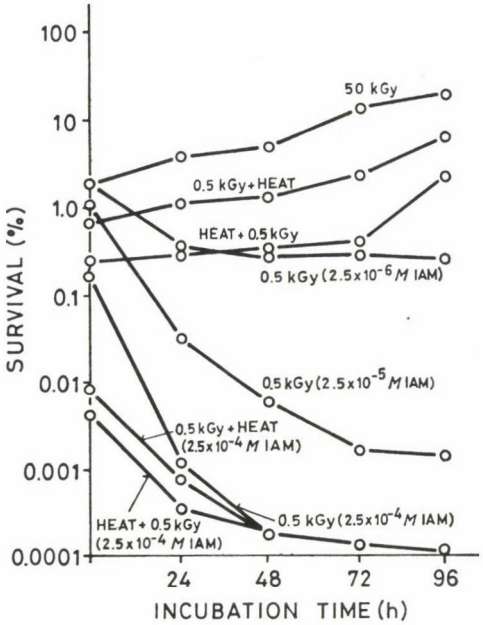


Fig. 4. Recovery profiles of irradiated and thermoradiated spores of *A. flavus* and same after sensitization with two concentrations of IAM. The aliquots from sensitized and unsensitized spore suspensions given radiation and combination treatment were diluted 100× in sterile distilled water and maintained on a rotary shaker at 25 °C/96 h. The spore count was determined every 24 h by SPC method. The plotted values are the averages of 6 replicates from two experiments

moradiated spores would be clear from the results depicted in Fig. 4. Possible mechanisms involved in the sensitization have been described earlier (BRIDGES, 1960; 1969). The lethal free radicals produced by the sensitizer molecules (BRIDGES, 1969) may react with the vital molecules and amplify the lesions caused by thermoradiation that probably affects the repair enzyme system which plays significant role in recovery (PADWAL-DESAI and co-workers 1979). The present study also suggests that the spores of *A. flavus* could be used as model system for investigating the repair mechanism in eukaryotes.

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Address of the authors:

Dr. Sharad Ramchandra PADWAL-DESAI
Ashok Sadashiv GHANEKAR
Arun SHARMA

Biochemistry & Food Technology Division,
Bhabha Atomic Research Centre
Bombay—400 085. India

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# MICRONUTRIENT CONTENT OF SOME CEREALS AND CEREAL PRODUCTS

A. SZALAY and A. MURÁNYI

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Results of a detailed microanalytical study are presented here, which might contribute data to a reliable assay of the changing micronutrient balance of human diet. The Mn, Cu and Zn content of wheat, rye, rice further of various wheat milling products and bakery products are investigated. With progressive refinement and decreasing ash content the Mn, Cu and Zn content is very significantly decreasing.

The contribution of cereal products to the Mn, Cu and Zn supply of human diet is very significant. In the affluent countries the cereal consumption decreases and the refinement of cereal products improves. As a consequence of these changes the Mn and Cu supply decreases. Zn is replenished by increasing consumption of meat and dairy products.

## 1. Materials and methods

Mn, Cu and Zn are very important micronutrients of which many essential biochemical roles in the living body are known and probably many others are awaiting exploration. Atomic absorption spectrometry was used for the analysis of Mn, Cu, Zn. Measurements of Fe, J, F, Co, etc. content were omitted here.

About 100 g of the grain samples were washed from dust and dried at 105 °C and ground to a powder. 2.5 g of this powder was ashed in an oven up to max. 550 °C. The ashes were dissolved in HCl and diluted to 0.1 *N* and the solution was sprayed into the acetylene flame of AAS. The results are expressed in ppm (mg/kg) of the dry weight.

Samples were collected with the intention to obtain reliable representative mean values. The number of parallel analyses and the sources of the samples are mentioned in the text.

Various bakery and dry pastry products were collected from different shops and at different times. The manager of the experimental mill of the State Trust of Cereal Industry, Hajdúnánás kindly milled and supplied us with a complete refinement series of flours from the same sample of wheat. The data contained in Figs. 1, 2, 3 originate from the analyses of these samples.

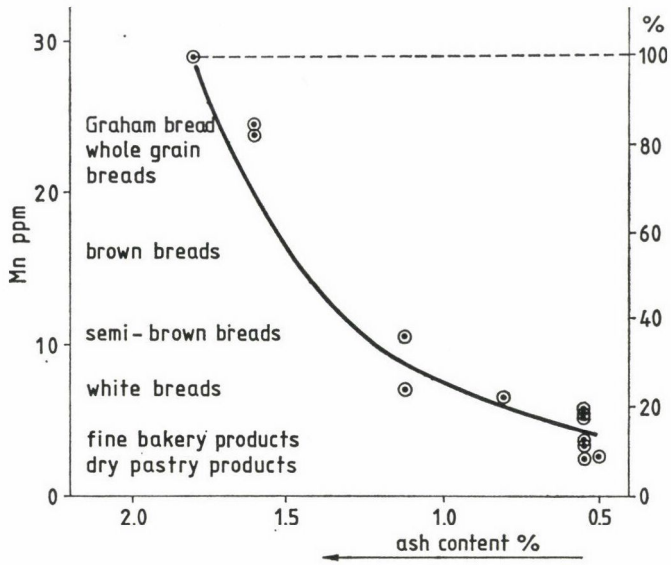


Fig. 1. Mn content of refined flour products of a wheat sample. Abscissa: Ash content in weight %; Ordinate – left: Mn content in ppm/dry weight, – right: Mn content in % of that of the original wheat sample

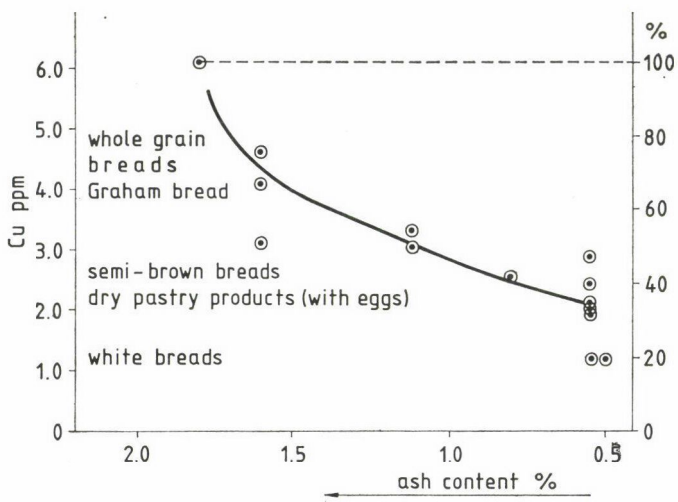


Fig. 2. Cu content of refined flour products of a wheat sample. Abscissa: Ash content in weight %; Ordinate – left: Cu content in ppm/dry weight, – right: Cu content in % of that of the original wheat sample

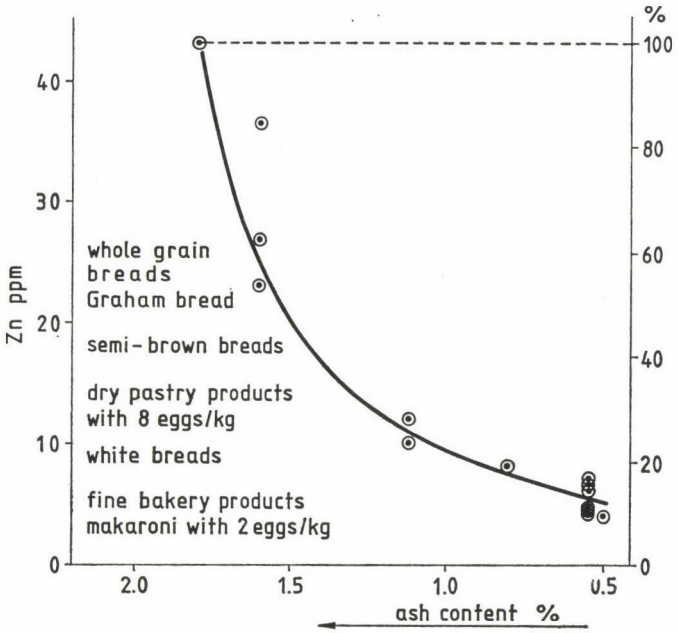


Fig. 3. Zn content of refined flour products of a wheat sample. Abscissa: Ash content in weight %; Ordinate – left: Zn content in ppm/dry weight, – right: Zn content in % of that of the original wheat sample

## 2. Results

### *2.1. Micronutrient content of wheat samples*

Table 1 demonstrates the micronutrient content of some winter wheat grains grown on soils of various quality. There is an influence of soil, as some bad soils impair the uptake of micronutrients. There might be some influence of the subspecies and weather of the season (amount and distribution of precipitation) so there is a natural variancy in the micronutrient content.

Data under No. 1 represent possibly low micronutrient content obtained by analyzing 12 samples from a low quality calcareous sand type of soil on which wheat growing is still profitable. Data under No. 2 represent 4 samples of wheat taken from very good quality black chernozem soils. Data under No. 3 represent a good quality black meadow chernozem soil.

As samples in Table 1 were chosen for comparison and somewhat arbitrarily biassed (low-grade and high-grade soils) we analyzed further 20 independent samples, from different places and soils, cultivated in large commercial quantities by major agricultural farms. They are represented in Table 2. They can be regarded statistically as random samples, averaged

Table 1

*Comparison of the micronutrient content of some winter wheat samples from various soils* (in ppm/dry weight)

| Origin | Mn | Cu | Zn |
|---|---|---|---|
| Deficient calcareous sand soil mean value of 12 samples | 22.1±0.50 | 4.31±0.11 | 21.5±0.60 |
| Rich black clayey (chernozem) soil, four different wheat subspecies samples | 35.2<br>32.3<br>31.2<br>37.3 | 4.70<br>3.80<br>4.20<br>4.00 | 25.9<br>20.4<br>25.5<br>24.1 |
| Black meadow (chernozem) soil | 26.7 | 4.05 | 19.1 |

from large quantities, blended in large silos. The intervals of standard deviation are given here with 68% confidence level of the mean values. Significant differences exist in the Mn content.

The mean values of this total set consisting of 20 samples can be regarded as reliable representation of the usual micronutrient content of wheat samples.

### *2.2. Micronutrient content of rye samples*

After wheat, rye is an important cereal for bread baking. Some rye flours are added to wheat breads. By the kind cooperation of the State Enterprises for Cereal Commerce and Mill Industries we obtained rye samples from large storing and blending silos with known geographical origin.

The results are indicated in Table 3 with the mean values and standard deviations of the 12 samples. It seems that the Mn content is somewhat higher and the Cu content is somewhat lower than that of the wheat samples of Table 2.

### *2.3. Micronutrient content of rice samples*

The greatest part of the population of the world is consuming rice as the most important staple food and energy source. Wheat is the second most consumed one. We analyzed a number of rice seeds and samples, because rice seems the most important source of micronutrients of the population of East, South-East and South Asia.

Table 2

*Micronutrient content of winter wheat samples*
(in ppm/dry weight)

| Origin of samples (Place, soil) | Mn | Cu | Zn | Ash content (weight %) |
|---|---|---|---|---|
| Vörös Csillag Cooperative, Balmazújváros (Hungary) | | | | |
| adobe soil | 48.0 | 3.49 | 21.3 | 1.74 |
| adobe soil | 43.0 | 3.71 | 26.1 | 1.62 |
| adobe soil | 41.2 | 3.51 | 21.1 | 1.70 |
| adobe soil | 37.0 | 3.61 | 22.8 | 1.70 |
| adobe soil | 35.8 | 3.18 | 20.9 | 1.77 |
| Mean value ± s | 41.0±2.19 | 3.50±0.09 | 22.4±0.97 | 1.71±0.02 |
| Vörös Csillag Cooperative, Hajdúböszörmény (Hungary) | | | | |
| adobe soil | 41.6 | 3.24 | 22.4 | 1.68 |
| adobe soil | 38.4 | 3.89 | 22.8 | 1.97 |
| adobe soil | 36.2 | 4.50 | 24.7 | 1.69 |
| adobe soil | 33.6 | 2.81 | 18.5 | 1.56 |
| adobe soil | 36.3 | 3.54 | 20.4 | 1.75 |
| Mean value ± s | 37.2±1.33 | 3.60±0.29 | 21.8±1.06 | 1.73±0.06 |
| State Farm, Hajdúszoboszló (Hungary) adobe-clay soil | | | | |
| adobe soil | 23.7 | 3.38 | 21.3 | 1.65 |
| adobe soil | 38.4 | 4.58 | 32.3 | 1.89 |
| adobe soil | 30.1 | 4.68 | 23.9 | 1.64 |
| adobe soil | 39.3 | 4.43 | 25.6 | 1.91 |
| adobe soil | 40.2 | 4.95 | 23.8 | 1.77 |
| Mean value ± s | 34.3±3.21 | 4.40±0.27 | 25.4±1.86 | 1.77±0.05 |
| Zöldmező Cooperative, Püspökladány (Hungary) | | | | |
| meadow, salty underground | 36.4 | 3.21 | 34.1 | 1.68 |
| meadow, salty underground | 32.2 | 5.34 | 34.8 | 1.88 |
| meadow, solonetz | 40.5 | 5.73 | 46.5 | 1.95 |
| meadow, salty underground | 42.9 | 5.31 | 47.8 | 2.05 |
| meadow, salty underground | 43.7 | 4.75 | 37.9 | 1.58 |
| Mean value ± s | 39.1±2.15 | 4.87±0.44 | 40.2±2.91 | 1.83±0.08 |
| Mean value of total ± s | 37.9±1.21 | 4.09±0.19 | 27.5±1.92 | 1.76±0.03 |

Table 3

*Micronutrient content of rye samples*
(in ppm/dry weight)

| Place of origin | Mn | Cu | Zn | Ash cont. (weight %) |
|---|---|---|---|---|
| Hajdúböszörmény | 49.2 | 3.71 | 26.6 | 1.71 |
| Hajdúböszörmény | 48.5 | 3.63 | 26.6 | |
| Hajdúböszörmény | 47.6 | 3.61 | 26.5 | 1.70 |
| Hajdúböszörmény | 48.3 | 3.67 | 26.5 | |
| Nyírábrány | 47.2 | 3.39 | 27.9 | 1.76 |
| Nyírábrány | 45.7 | 3.39 | 27.4 | |
| Nyírábrány | 44.4 | 3.34 | 26.8 | 1.73 |
| Nyírábrány | 45.9 | 3.39 | 27.4 | |
| Nyíradony | 41.6 | 3.91 | 27.1 | 1.59 |
| Nyíradony | 41.8 | 3.71 | 27.9 | |
| Nyíradony | 41.8 | 3.69 | 28.6 | 1.56 |
| Nyíradony | 41.7 | 3.79 | 27.8 | |
| Mean value $\pm$ s | 45.3$\pm$0.86 | 3.60$\pm$0.05 | 27.3$\pm$0.20 | 1.68$\pm$0.03 |

It can be seen from Table 4 that brown rough rice grains have a much higher manganese content than wheat or rye grains. The table demonstrates also some commercial rice samples of the following grades: unpolished rice, polished rice, pre-cooked (and dryed) rice. Further, we investigated home cooked polished and unpolished rice. They preserved the original micronutrient content, however the cooking technique was the following: 1 volume of rice boiled in 3 volumes of water and slowly cooked until the whole water was taken up by the rice and then it was cooked till tender.

By comparing the micronutrient content of polished or unpolished rice samples ready for consumption, it can be seen that their micronutrient content is much higher than that of fine wheat and rye products. It seems that a population consuming large quantities of rice is better supplied with Mn, and perhaps even with Zn than populations consuming wheat or rye.

### *2.4. Micronutrient content of wheat products*

The processing of wheat by the mills impairs the micronutrient content of flours. As is well known, a large part of mineral substances and micronutrients are concentrated in the husk and germ of the grains. The technical development

Table 4

*Micronutrient content of rice samples*
(ppm/dry weight)

| Sample | Mn | Cu | Zn |
|---|---|---|---|
| Brown, rough rice | 55.8 | 1.60 | 20.9 |
| (rice in the husk) | 49.4 | 1.20 | 21.3 |
| | 51.2 | 0.96 | 19.9 |
| Mean value ± s | 52.1±1.91 | 1.25±0.19 | 20.7±0.42 |
| Unpolished rice | 10.8 | 1.50 | 25.3 |
| (white) | 12.0 | 1.40 | 26.2 |
| | 11.5 | 1.52 | 24.8 |
| | 13.0 | 1.38 | 23.0 |
| | 11.8 | 1.53 | 27.0 |
| | 9.9 | 1.67 | 25.5 |
| Mean value ± s | 11.5±0.43 | 1.50±0.04 | 25.3±0.56 |
| Home cooked and dryed | 12.2 | 1.67 | 21.0 |
| unpolished rice | 10.7 | 1.42 | 17.1 |
| Home cooked and dryed polished rice | 16.2 | 1.93 | 18.1 |

of milling industry efficiently separates the bran and germs from the various kinds of flours and the micronutrient content of flours is depleted. This is known and was investigated already by CZERNIEJEWSKI and co-workers (1964).

Whiter flours are regarded as finer products commercially. The grade of refinement of cereal products is standardized in different countries in a different way. Flours of increasing grade of refinement are produced after crushing and separation of bran and germs by sifting. Refined flour of whiter appearance has a decreased mineral and ash content. Ash content of wheat grains approximates 2% (about 1.8% in the average) and the finest flours have an ash content of about 0.5%. If 77 weight % flour is extracted from wheat then the ash content is about 0.8%. This is a brown flour used for bread baking. From wheat about 72% fine white flour of 0.5% ash can be extracted. This is called in the USA patent flour or fine white flour.

The ash content consists mainly of macroelements P, K, Mg, Ca. The contribution of micronutrients (Mn, Zn, Cu) is small, less than 0.004th part of the whole ash weight. The micronutrient content is correlated with the macronutrient mineral elements and decreased with decreasing ash content.

Table 5

*Influence of milling and refining on the micronutrient content of cereal products*
(ppm/dry weight)

Hungarian Standard Specification of flours is related to ash content, *e.g.*:
BL 80 is a wheat flour for bakery bread with 0.80% ash
BL 55 is a wheat flour for fine bakery products with 0.55% ash
TL, 50 is a wheat flour for pastry products with 0.50% ash
BFF 55 is a wheat flour for biscuits, cakes, sweet pastry, etc. with 0.55% ash
RL 90 is a rye flour for brown rye bread with 0.90% ash
BL 112 is a wheat flour with 1.12% ash, for brown wheat bread and additive blending component for bakery products

| Serial No. | Sample | Extr. yield (%) | Mn | Cu | Zn | Ash cont. (weight %) |
|---|---|---|---|---|---|---|
| 1 | Wheat before sifting from Hajdúnánás | | 29.0 | 6.08 | 43.1 | 1.80 |
| 2 | Wheat No. 1, after purifying by abrasion before crushing | | 23.8 | 4.08 | 36.4 | 1.60 |
| 3 | Wheat from Debrecen, after purifying and before crushing | | 24.5 | 3.08 | 26.8 | 1.60 |
| 4 | Wheat from Debrecen, after purifying and before crushing | | 24.5 | 4.60 | 23.0 | 1.60 |
| | Cereal products from Serial No. 1 | | | | | |
| 5 | bran of No. 1 | 22.6 | 59.2 | 10.6 | 85.6 | |
| 6 | germ of No. 1 | 0.4 | 76.4 | 10.4 | 162.0 | |
| 7 | flour BL 80 of No. 1 | 42.0 | 6.52 | 2.56 | 8.44 | 0.80 |
| 8 | flour BL 55 of No. 1 | 26.5 | 3.44 | 2.44 | 4.32 | 0.54 |
| 9 | flour BFF 55 of No. 1 | 1.0 | 2.52 | 1.20 | 4.92 | 0.53 |
| 10 | flour TL 50 of No. 1 | 7.0 | 2.64 | 1.20 | 4.20 | 0.48 |
| 11 | semolina of No. 1 | 0.5 | 3.20 | 2.28 | 4.84 | 0.55 |
| | Cereal products from Serial No. 3 and 4 | | | | | |
| 12 | bran of No. 3 and 4 | 22.6 | 60.4 | 9.04 | 79.2 | |
| 13 | germ of No. 3 and 4 | 0.4 | 95.6 | 7.72 | 122.0 | |
| 14 | flour BL 112 of No. 3, 4 | | 7.12 | 3.36 | 10.1 | 1.10 |
| 15 | flour BL 55 of No. 3, 4 | 26.5 | 3.72 | 1.92 | 4.96 | 0.54 |
| 16 | semolina of No. 3, 4 | 0.5 | 4.20 | 1.96 | 4.84 | 0.55 |
| 17 | bran from the market | | 83.3 | 12.6 | 65.5 | |
| 18 | fodder flour from the market | | 52.4 | 7.32 | 53.0 | |
| 19 | flour BL 112 from the market | | 10.6 | 3.02 | 12.2 | 1.11 |
| 20 | flour BL 55 from the market | | 5.52 | 2.89 | 6.88 | 0.52 |
| 21 | flour BL 55 from the market | | 5.54 | 2.15 | 7.22 | 0.51 |
| 22 | flour BL 55 from the market | | 5.29 | 2.07 | 6.73 | 0.49 |
| 23 | semolina from the market | | 7.70 | 1.50 | 9.40 | 0.55 |
| 24 | rye bread flour RL 90 | | 9.46 | 1.63 | 8.37 | 0.87 |

We carried out a detailed analysis on a certain wheat sample and cereal products prepared from it in the experimental mills of the State Trust of Cereal Industry, Hajdúnánás and Debrecen, Hungary.

Table 5 demonstrates the analytical data of these cereal products and some additional data obtained by the analysis of commercial products from the market further one rye flour sample.

On the top of Table 5 the standard specifications by ash content and the main usage is listed. Table 5 contains in addition of the Mn, Cu and Zn content in ppm/dry weight the ash content determined in our laboratory in weight % and the extraction yield of the experiment as determined by the mill.

It should be noted here that the extraction yield in this table is not identical with the maximum possible extraction yield obtainable from the same quantity of wheat because in this special experiment a whole series of extraction products have been separated from sample number 1. It is apparent from these data in Table 5 that already the first step of treatment of wheat, the purification from dust and soil, etc. by abrasion decreases the micronutrient content significantly. (Sample No. 2.) It seems that in addition to dust, some of the husk is derased and micronutrient content is lost.

After crushing, bran and germs are separated by sifting before further milling. It is apparent in Table 5 that the Mn, Cu and Zn content in germ and bran is very high and by the separation of these by-products much of the micronutrient content is lost from the human diet, however it is utilized in animal fodder. (N.b.: some germ preparations are utilized as food, flakes, baby food, *etc.*)

The refined cereal products utilized in the human diet are very depleted in Mn, Cu and Zn.

Figures 1, 2 and 3 demonstrate the loss of Mn, Cu and Zn as a function of refinement, i.e. decreasing ash content.

Figure 1 demonstrates the Mn content of refined cereal products and the same wheat from which they were extracted. The abscissa is the ash content, the ordinate on the left is the Mn content in actual ppm/dry weight, and on the right side the decrease of it in percentage compared to the content of the wheat taken as 100%.

It can be well seen in Fig. 1 that by progressing milling refinement and decreasing ash content the micronutrient content of the cereal products decreases very significantly, so that the micronutrient content of 72% extraction yield patent flour (fine white flour) amounts only to about 14% of the Mn content of the wheat.

In Fig. 2 similarly the decrease of Cu content of cereal products is represented on the ordinate, to the left in ppm, and to the right in percentage of the total Cu content of the original wheat. In fine grade wheat flours only about 1/3 of Cu content remained.

In Fig. 3 the decrease of Zn content is demonstrated. The decrease of Zn content is as significant as that of Mn. No more than about 1/8 part of Zn concentration remains in the refined white flour.

### *2.5. Micronutrient content of bakery and dry pastry products*

The micronutrient content of bakery and dry pastry products depends on the content of the original flours and the proportion in which they are blended.

No conclusion can be drawn from the ash content of breads because salt and in some countries some macronutrient minerals are added to the dough

Table 6

*Micronutrient content of bakery and dry pastry products*

| Serial Nr. | Product | Mn ppm in | | Cu ppm in | | Zn ppm in | |
|---|---|---|---|---|---|---|---|
| | | fresh | dry | fresh | dry | fresh | dry |
| 1 | Graham bread* | 16.2 ±0.62 | 22.5 1.16 | 1.84 0.05 | 2.55 0.11 | 9.80 0.11 | 13.6 0.42 |
| 2 | Semi-brown bread (mixed wheat-rye bread) | 6.39 ±0.40 | 9.93 0.62 | 1.47 0.13 | 2.28 0.21 | 6.96 1.11 | 10.7 1.63 |
| 3 | Semi-brown bread (mixed wheat-rye bread) | 4.70 ±0.05 | 7.10 0.07 | 1.50 0.04 | 2.30 0.06 | 11.9 0.51 | 17.9 0.80 |
| 4 | Rye bread (mixed rye-wheat bread) | 4.50 ±0.10 | 6.80 0.12 | 1.80 0.06 | 2.70 0.08 | 12.2 0.11 | 18.4 0.17 |
| 5 | White wheat bread | 5.15 ±0.09 | 7.61 0.13 | 0.62 0.04 | 0.92 0.06 | 6.48 0.13 | 9.59 0.20 |
| 6 | Fine bakery products (bun, roll) | 2.97 ±0.05 | 4.03 0.06 | 0.74 0.04 | 1.00 0.06 | 4.27 0.10 | 5.78 0.14 |
| 7 | White wheat loaf (refined wheat flour) | 1.90 ±0.10 | 2.80 0.14 | 0.60 0.05 | 0.90 0.08 | 2.40 0.13 | 3.60 0.19 |
| 1 | Macaroni (with 2 eggs/kg) | 1.50 ±0.06 | | 1.80 0.07 | | 5.20 0.16 | |
| 2 | Granulated dry pastry (with 4 eggs/kg) | 1.70 ±0.10 | | 2.20 0.11 | | 6.70 0.09 | |
| 3 | Noodles for soup (with 4 eggs/kg) | 1.30 ±0.03 | | 1.70 0.10 | | 6.50 0.13 | |
| 4 | Noodles for soup (with 8 eggs/kg) | 2.10 ±0.07 | | 2.00 0.14 | | 15.8 0.33 | |
| 5 | Noodles for soup (with 8 eggs/kg) | 1.60 ±0.13 | | 1.80 0.07 | | 12.0 0.43 | |

* Graham bread obtainable and analyzed here differs from standard Graham bread. It contains some white flour, blended to 99% extraction whole wheat flour (see text).

before baking. However the micronutrient content can be determined analytically as in the cereal products or it can be calculated if the micronutrient content of the flours from which it was blended is known. As breads are sold and consumed in a fresh wet state it is necessary to indicate the micronutrient content related to the fresh and to the dry weight as well.

A number of Hungarian standard bread and dry pastry products have been analyzed in this work and the results are indicated in Table 6. From each product 6 parallel samples have been analyzed and the mean values and s.d. are demonstrated in the table.

The following explanations are necessary for bread and dry pastry products in Table 6. Standard *Graham* bread or whole grain wheat bread contains the total amount of micronutrients of the grains except the loss due to the purifying abrasion. *Graham* bread obtainable here and analyzed in this work under Serial No. 1, is however a mixed bread, blended from 45.6% *Graham* flour of 99% extraction yield wheat flour and 30.4% BL 55 white wheat flour, to which salt, yeast and water are added up to 100%.

Bread products of the following serial numbers are made from blended flours. No. 2 semi-brown bread contains 85% wheat flour (BL 112) and 15% rye flour (RL 90); No. 3 semi-brown bread is baked from 70% wheat flour (BL 112) and 30% rye flour (RL 90) No. 4 rye bread is produced from 70% rye flour (RL 90) and 30% wheat flour (BL 112). No. 5 white wheat bread is produced from wheat flour (BL 80). This is the bread consumed predominantly in Hungary. No. 6 fine bakery products (buns, rolls of bread, *etc.*) are produced from wheat flour (BL 55). No. 7 white wheat loaf is produced from white wheat flour (BL 55). This is similar to the 72% extraction flour, patent flour in the USA.

The analytical data of dry pastries in Table 6 are of course related to the ready products and so the micronutrient content is enhanced by the addition of eggs. This is particularly noticeable in the case of Zn. Pastry products are made of fine wheat flours of an ash content of 0.50%, *e.g.* TL 50 wheat flour.

## 3. Conclusions

From the overall picture presented by this study the following facts can be emphasized.

Cereals and cereal products are very abundant sources of Cu, Mn and Zn micronutrients. Whole grain wheat breads and *Graham* bread preserve practically the whole micronutrient content. However in the course of milling and refining processes very much of the micronutrients are lost from the human diet because they are concentrated in the bran and germ. Rice is an exception, because it contains significant quantities of Mn and Zn, even in the white polished form.

Although exact, comparable earlier statistical data do not exist, it seems that the present total cereal consumption amounts to hardly more than 1/3 of that towards the end of the 19th century and it consists largely of fine white cereal products. Large consumption of sugar, fats, oils, replace or increase the calory content of food without any micronutrient and minerals. Animal proteins (meats, dairy products, *etc.*) are poor in Mn and Cu, however they replenish the missing Zn content.

As the diet of the affluent countries shifted in the course of the recent century towards less cereals with white flour products preferred, the diet of the population became depleted from the large part of Mn and Cu micronutrients. Zn supply is replenished by the high Zn content of dairy products and meats (animal proteins) the consumption of which increased very significantly in the affluent countries during the same time. These problems will be treated in more detail in subsequent publications. It is well known that the consumption of cereals decreased very substantially in the course of this century.

This great shift in nutritional habits of the affluent nations emphasizes the question whether the decreased supply in Mn and Cu micronutrients which are essential for life, health and proliferation are not influencing the general health status of these populations in a statistical sense.

This study was carried out with the intention to supply reliable data for further enquiry into this complex problem.

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Address of the authors:

Dr. Alexander SZALAY
Dr. Annamária MURÁNYI

Institute for Nuclear Research of the Hungarian Academy of Sciences
H-4001 Debrecen, Bem tér 18/c. Hungary

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# PROBLEMS RELATED TO THE RESIDENCE TIME OF FOOD MATERIALS IN PROCESSING EQUIPMENT; EXAMPLES FROM THE CANNING INDUSTRY

I. KÖRMENDY

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The paper attempts to interpret the residence time as a probability variable, when the processed material consists of solid pieces, or can be treated as a continuum or belongs to disperse systems. The operational character of the processing equipment (stationary, quasi- and pseudo-stationary, non-stationary) is analysed in connection with the residence time as a probability variable. A description is given of a mechanical model, which can be substituted, in view of the residence time, for the technological equipment. This model is also of didactic importance. Knowledge related to units connected in series or in parallel, as well as to the feed-back type system, is discussed. The problem of dependence and independence in serially connected units is also investigated. The basic principles of three measurement methods, and a method of correction related to the distribution or density function as obtained from the measurements, are also studied.

Finally, the results of measurements hitherto carried out in the canning industry in Hungary are summarized.

The aim of this paper was to present a comprehensive study on the subject. An attempt has been made to synthesize a number of different results, but in the treatment of the subject the paper follows original ideas and design. Original solutions are presented for some of the problems, though it is possible that other authors have independently arrived at the same conclusions. Monographs on the subject of residence time are to be found in the literature on reactor technology (LEVENSPIEL, 1962). However, the conception of the author differs from these in many respects. As regards the operational character of the equipment, the grouping of RANDALL and co-workers (1971) was adopted. The concept of the element or elemental part was based on canning industrial practice. Definitions for residence time as a probability variable, their application to materials used in the canning industry, the model substituted for the equipment, the results pertinent to the average residence time in stationary, quasi-stationary and special pseudo-stationary (see para. 1.4) cases, simple cases of linear dependence and correlation, and the methods of correction suggested for the evaluation of the measurements, are original conceptions of the author.

The problem of residence time has acquired importance in consequence of the increasing application of reaction kinetics (LENZ & LUND, 1980), physics and biology in the food industry. At the present level of computer techniques the treatment of these subjects has become possible even for indeterminate

residence times. Optimization tasks can be resolved on the basis of the above (NORBACK, 1980). By analysing the distribution function of residence time the operation of an equipment can be examined and its defects discovered (WOODROW, 1978). Data required for the automated control of the equipment can also be obtained. A knowledge of the distribution function of residence time is indispensable when dimensioning sterilizers and pasteurizers, where the processed material passes through the unit in a continuous way (SCALZO *et al.*, 1969; CHARM, 1971). Residence time must be taken into account in the field of fruit and tomato juice concentration (MEFFERT, 1964; KÖRMENDY, 1980), in sugar beet extraction, and in saccharose degradation (SCHLIEPHAKE *et al.*, 1975; BALOH, 1979).

## 1. Theoretical considerations

### *1.1. Residence time as a probability variable*

Equipment may have determinate or indeterminate residence time. In equipment with determinate residence time, the residence time varies between relatively narrow limits around a predetermined mean value. In equipment with indeterminate residence time, it is not known how long a selected element of the input material may remain in the equipment. In this case, residence time is a probability variable and therefore has a distribution or density function.

It is an important criterion of elements or elemental particles that, no matter how their physical and chemical properties may change in the equipment, theoretically they can always be identified with the element introduced or removed. Thus, the element may be labelled with an individual symbol. The number of elements introduced and removed has to be identical. The concept of an element is not bound to its size. It may be of macroscopic size (*e.g.* one pea, an apple slice, a cucumber) or microscopic (*e.g.* a plant cell) or even of submicroscopic size (*e.g.* a molecule). In a material considered as a continuum the formation of elements having infinitesimal size is possible.

In the case of an element of finite size, the residence time is the residence time of a well defined point of the element, expediently that of the centre of the mass.

If comminution or integration occurs in the equipment, then the smaller particles should always be considered as elements (subsequent to comminution or previous to integration).

The utilization of the concept of the element is also expedient because it is a chemical, physical and often biological unit and as such, forms the subject of study, quality testing or processing.

The distribution function of residence time as a probability variable (marked: $\tau$) is interpreted as follows: two fixed points of time are chosen from

the operation time of the equipment $u_1$ and $u_2$ ($u_2 > u_1$). The distribution function gives the proportion of the number of elements introduced into the equipment between the two points of time which reside for a period shorter than a chosen $t$ time (Basic Definition 1). Another similar but not identical definition takes into account the out-going elements instead of those introduced (Basic Definition 2). Only under the fulfilment of certain conditions do the two definitions lead to identical distribution functions.

From the distribution functions derived from the basic definitions, various further distribution functions can be derived. In practice, the most important of these, are shown in Table 1.

As an example, Definition 2 may be interpreted in the following way, if Basic Definition 1 is taken into account: the distribution function shows the proportion of the mass of material entering the equipment between the two fixed points of time $u_1$ and $u_2$ ($u_2 > u_1$), that remains in the equipment for a period shorter than the predetermined $t$ residence time.

Under the fulfilment of certain conditions the derived distribution functions may be equivalent to one another and to that of the basic definition, while they differ in their contents (the special conditions are given in Table 1).

Based on industrial practice two major types of material can be distinguished: those of discrete pieces and those forming a continuous system. The latter type is frequently treated as a continuum. If the material is considered as a continuum, the properties under examination can be regarded as continuous and differentiable functions of the space and time coordinates. In the canning industry materials which may be classified as rough disperse systems are frequently encountered. These are continuous systems, however, containing larger pieces dispersed in a suspension of smaller particles (*e.g.* pulps, jams).

In materials consisting of discrete pieces (particles), the pieces themselves are the elements. The derived distribution or density functions can be easily determined from the distribution or density function given by the basic definition. The mass, the mass of dry matter or the volume of each element may be measured and the appropriate formula in Table 1 can thus be applied.

To divide a continuum into its elemental parts one has to proceed from the physical, chemical or biochemical content of the process under examination. Thus, a continuum may be reduced to infinitesimal elements of identical mass, dry matter mass or volume. The corresponding derived distribution function, based on mass ratio, dry matter mass ratio or volume ratio, will be equal in each case to the distribution function based on the number of elements. Therefore, in relation to a continuum, greater freedom is enjoyed, though, the competence required is also greater than with materials consisting of discrete pieces. The following volume integrals are valid, when the volume (V) is parti-

Table 1

*Distribution and density functions belonging to the residence time as a probability variable*
Symbols are listed at the end of the text

| Serial number | Type of definition | Interpretation of the distribution function | Dimensions | Symbols of the functions and conditions of equality | Equations to calculate the density function |
|---|---|---|---|---|---|
| 1a | Basic Definition 1 | Ratio of the number of elements at inlet | Number of elements/number of elements | $F(t) = P(\tau < t)$ | $f(t) = \frac{\mathrm{d}F}{\mathrm{d}t}$ |
| 1b | Basic Definition 2 | Ratio of the number of elements at outlet | | | |
| 2 | Derived distribution | Mass ratio | kg/kg | $F_m = F$, if $m = \overline{m}$ | $f_m(t) = \frac{m}{\overline{m}} f(t)$ |
| 3 | functions | Dry matter mass ratio | kg/kg | $F_s = F$, if $am = \overline{am}$ | $f_s(t) = \frac{am}{\overline{am}} f(t)$ |
| 4 | | Volume ratio | $m^3/m^3$ | $F_v = F$, if $\frac{m}{\varrho} = \overline{\left(\frac{m}{\varrho}\right)}$ at any $t$ | $f_v(t) = \frac{m/\varrho}{\overline{(m/\varrho)}} f(t)$ |

Additional formulae $m = \frac{1}{n}\sum_{j=1}^{n} m_j \qquad am = \frac{1}{n}\sum_{j=1}^{n} a_j m_j \qquad \frac{m}{\varrho} = \frac{1}{n}\sum_{j=1}^{n} \frac{m_j}{\varrho_j}$

Interpretation and dimensions $m_j$ = mass of element, kg; $a_j m_j$ = mass of dry matter in one element, kg;

$\frac{m_j}{\varrho_j}$ = volume of one element.

$n$ is the number of elements entering or leaving between points of time $t - \mathrm{d}t/2$ and $t + \mathrm{d}t/2$

tioned into infinitesimal volumes (dV), not necessarily of equal size:

$$\int_{(V)} \varrho \, dV = m, \quad \int_{(V)} a\varrho \, dV = s, \quad \int_{(V)} dV = V \tag{1}$$

It should be noted, however, that the strict interpretation of the reduction of a continuum to its elements is encumbered by difficulties not treated here.

Let us now discuss a rough disperse system. In this case the proper proceeding is to divide the system theoretically into its components and determine their distribution functions individually. Any derived distribution function can be calculated from the individual functions on the basis of rules related to the distribution of mixtures. A pulp, for instance, may be divided into parts consisting of rough pieces (of macroscopic size), finely comminuted fibrous particles (of microscopic size) and a material considered as a continuum, which is an aqueous solution of submicroscopic particles.

### *1.2. Classification of processing equipments*

Processing equipments can be grouped in different ways, for instance: type of change in the processed material, type of unit operation, number of inlet and outlet connections, operational character of the equipment. Grouping according to the operational character is presented in Fig. 1 and Table 2. Grouping according to the number of inlet and outlet connections in view of residence time, with examples including process engineering character, is shown in Table 3.

Non-stationary operational character is exclusive for batch systems and it also characterizes the start and stop period of equipments with continuous

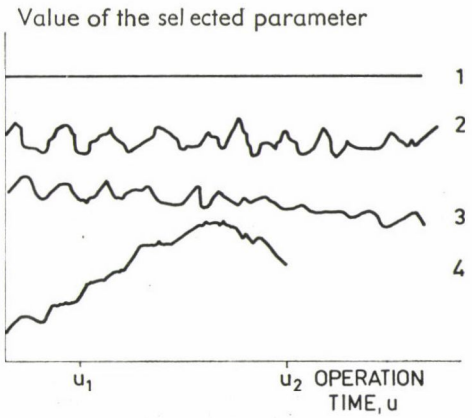


Fig. 1. Operational character of equipment with continuous operation 1: stationary; 2: quasi-stationary; 3: pseudo-stationary; 4: non-stationary

Table 2

*Grouping of the equipment according to the character of operation*

| Equipments with determinate residence time | |
|---|---|
| Character of operation | Example |
| Batch operation | Horizontal retort |
| Continuous operation | Hydrostatic sterilizer |

| Equipment with indeterminate residence time (Fig. 1) | |
|---|---|
| Character of operation | Example |
| Batch operation, always non-stationary (unsteady-state) | Batch-type evaporator |
| Continuous operation: | |
| Stationary (steady-state) | Theoretical (marginal) case |
| Quasi-stationary (quasi-steady-state) | Self-discharging centrifugal separator |
| Pseudo-stationary (pseudo-steady-state) | Continuously operating evaporator in the equilibrium condition |
| Non-stationary (unsteady-state) | Continuously operating evaporator at start and stop periods |

Table 3

*Grouping of equipments according to the number of inlet and outlet connections for the materials examined, in relation to residence time. The pertinent process engineering character of the example is also presented*

| Number of inlet and outlet connections | Process engineering character | Examples and notes |
|---|---|---|
| Single inlet, single outlet | Heat transfer | Pea blanching (cooking). The residence time of the blanching water is of no interest<br>Tomato concentration. Residence time of the vapour is of no interest |
| Single inlet, several outlets | Grading | Grading of peas according to size in grading drums |
| | Mechanical separation | Centrifugal separation in order to reduce the fibre content of tomato juice. Residence time of both the separated fibres and the juice with reduced fibre content is taken into account |
| Several inlets, single outlet | Mixing | Concentration of juices of different fruits to produce a mixed fruit juice concentrate |
| Several inlets, several outlets | Mass transfer | Production of apple juice by diffusion. The residence time of both the apple slices and the extraction water is of interest |

operation. An extensive variation of parameter values is characteristic (usually unidirectional) here.

Stationary (steady-state,) quasi-stationary (quasi-steady-state) and pseudo-stationary (pseudo-steady-state) character is interpreted according to RANDALL and co-workers (1971). In the stationary, theoretically marginal case every parameter is constant in time. Points of time $u_1$ and $u_2$ can be selected at will with respect to the $F(t)$ distribution function. In relation to the interval $u_2-u_1$, a lower limit value only has to be accounted for in the case of materials containing discrete pieces. In the case of quasi-stationary operation the parameters fluctuate around constant time averages. Thus, the interval $u_2-u_1$ has to be sufficiently great to bridge over fluctuations in time, but otherwise $u_1$ and $u_2$ may be chosen at will.

In the case of pseudo-stationary operation the values of the parameters or their averages over time suffer slow, unidirectional change. The interval $u_2-u_1$ has to be great enough to bridge over possible, relatively short fluctuations. In addition, the values $u_1$ and $u_2$ have to be carefully selected because, similarly to the non-stationary case, the $F(t)$ distribution function depends on both points of time.

### *1.3. Mechanical model which can be substituted for the equipment from the point of view of residence time*

The model developed by the author (KÖRMENDY, 1980) is shown in Fig. 2. The essence of the model is that the equipment is replaced by parallel conveyor belts with determinate residence times. The input flow of material branches out on the individual belts and is reunited again on leaving the belts (single inlet and outlet connection). It is not known in advance which belt will forward any particular element.

In Fig. 2 the number of belts is finite and the density function is step-wise in shape. By increasing the number of belts unlimitedly, continuous distribution and density functions are obtained (Fig. 3). The velocity of the belts is constant; thus, the length of a belt is proportional to the residence time on the belt.

The tubular model, earlier applied by the author (KÖRMENDY, 1974), can only be used with materials of the continuum type and only under the observation of specified conditions. The model shown in Fig. 2 is applicable to any of the materials mentioned in para. 1.1, also when changes in their mass (drying, concentration, increase in mass) occur in the equipment.

The hatched area under the step-wise density function represents the product $f_i \Delta t$ which gives the number of elements entering a belt with serial number $i$ and average residence time $t_i$, within the time interval $u_2-u_1$, related to the total number of elements entering in the same time interval, in agree-

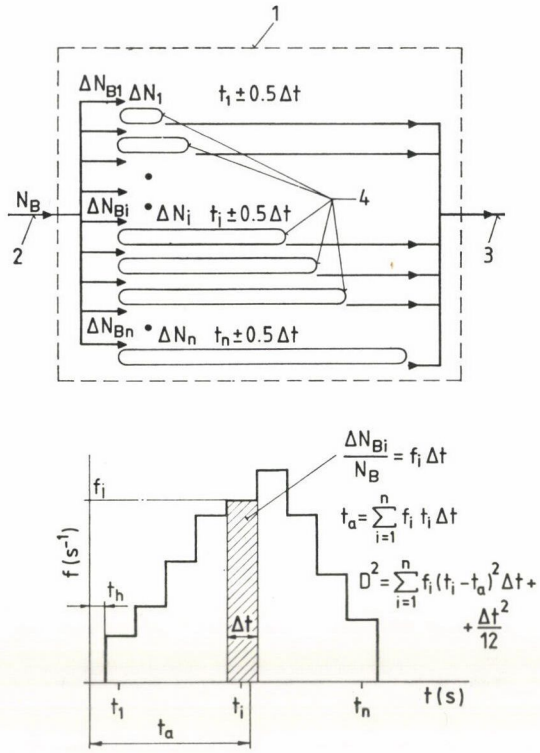


Fig. 2. Model replacing the equipment from the point of view of residence time and the pertinent density function. 1: symbolic boundary of the equipment; 2: material entering the equipment; 3: material leaving the equipment; 4: belts with determinate residence time, the speed of the belts is considered constant

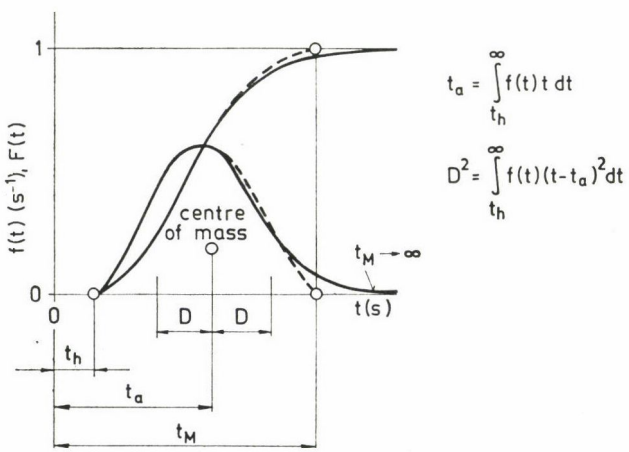


Fig. 3. Characteristic distribution and density function of the residence time as a probability variable. $t_a$, D relates to the case illustrated by the solid line

ment with Basic Definition 1. The distribution function has four main characteristics: the dead-time ($t_h$), the maximum residence time ($t_M$), the expected value ($t_a$) and the dispersion ($D$). These are shown in Figs. 2 and 3.

Similar characteristics may be developed for the other density functions listed in Table 1 ($t_{ma}$, $D_m$, $t_{sa}$, $D_s$, $t_{va}$, $D_v$). The dead-time and the maximum residence time are identical both in the case of the basic definition and for the derived distribution functions, thus the same symbol may be used for them.

### *1.4. Conclusions related to the expected value of residence time*

Based on the model described in Section 1.3., at belt $i$

$$\Delta N_{Bi} = N_B f_i \Delta t \tag{2}$$

In the stationary case, since the elements enter the belt at a constant rate: the number of elements which are present simultaneously on the belt is

$$\Delta N_i = \frac{\Delta N_{Bi}}{u_2 - u_1} \cdot t_i \,. \tag{3}$$

Substituting Equation (2) into Equation (3) and summarizing the number of elements present on individual belts, the total number simultaneously present in the equipment is

$$N = \sum_{i=1}^{n} \Delta N_i = \frac{N_B}{u_2 - u_1} \sum_{i=1}^{n} f_i t_i \Delta t \tag{4}$$

The expression beginning with the symbol of summation is the expected value of the distribution, denoted with $t_a$; thus Equation (4) leads to

$$t_a = \frac{N}{N_B/(u_2 - u_1)} \tag{5}$$

In the stationary case, therefore, the expected value of the residence time (also the average residence time) is obtained by dividing the total number of elements simultaneously present in the equipment by the number of elements entering (or leaving) in unit time. For a continuous distribution function the expression with the summation symbol is substituted by the corresponding integral in Fig. 3. If the distribution function based on the mass ratio is taken into account (from Table 1), then based on considerations similar to those above:

$$t_{ma} = \lambda_{ma} \cdot \frac{G}{G_B/(u_2 - u_1)} \tag{6}$$

Here, $\lambda_{ma}$ is a dimensionless value and shows from how much entering material the amount present simultaneously in the equipment originated. $\lambda_{ma}$ itself is an average value, since each of the belts may possess a different $\lambda_m(t)$, even in a stationary case. Thus for a continuous distribution function

$$\lambda_{ma} = \frac{t_{ma}}{\int_0^\infty \frac{f_m(t) \cdot t}{\lambda_m(t)} \cdot \mathrm{d}t} \tag{7}$$

The definition of $\lambda_m(t)$ is:

$$\lambda_m(t) = \frac{\mathrm{d}G'}{\mathrm{d}G} \tag{8}$$

where $\mathrm{d}G$ is the mass of material simultaneously present in the equipment and with a residence time between $t + \mathrm{d}t/2$ and $t - \mathrm{d}t/2$, while $\mathrm{d}G'$ is the mass of the same material when entering the equipment.

Rules in connection with $\lambda_{ma}$ in brief: Decrease in the mass in the equipment (concentration, drying, *etc.*):

$$\lambda_{ma} > 1$$

Constant mass:

$$\lambda_{ma} = 1$$

Increase in the mass (blanching, steeping in syrup):

$$\lambda_{ma} < 1$$

The value of $\lambda_{va}$ may be similarly formed from the distribution function of volume ratio according to Table 1. The results obtained for stationary operations may be applied to quasi-stationary operations as well, provided that $u_2 - u_1$ is sufficiently large. In the case of pseudo-stationary or non-stationary operations the above results cannot be used. For a special case of pseudo-stationary operation, when, as shown in Fig. 4, the amount of material entering the equipment in unit time decreases in a linear way, the following result may be deduced:

$$G = \frac{Q_{Ba} + Q_{Bb}}{2} \cdot t_{ma} + \frac{1}{2}(Q_{Ba} - Q_{Bb}) \frac{D_m^2 + t_{ma}^2}{u_2 - u_1} . \tag{9}$$

$G$ stands for the average value of the mass of material present simultaneously in the equipment between points of time $u_2$ and $u_1$. It can be seen that the

variance is also present in the relation ($D_m^2$). In Equation (9) the distribution function according to mass ratio (entering material) and $\lambda_{ma} = 1$ were taken into account.

### *1.5. Apparatus connected serially or in parallel, and feed-back systems*

In the case of several units in an equipment connected serially or in parallel or arranged in a feed-back system, the answer to two correlated questions was sought: how should the resulting distribution and density functions be calculated and what is the relationship between individual residence times as probability variables.

There are three different cases regarding serially connected units in an equipment:

- completely independent units;
- stochastically related units;
- functionally related units.

The mathematical formulae relate to the connection scheme in Fig. 5, taking into account Basic Definition 1.

The formulae were summarized in Table 4. Here, the stochastic relationship refers only to linear correlations and the functional relationship only to linear functions. Formulae with the designation "density function" relate to continuous distribution functions.

In the case of complete independence the rules relating to the addition of independent probability variables are valid. The greater the number of

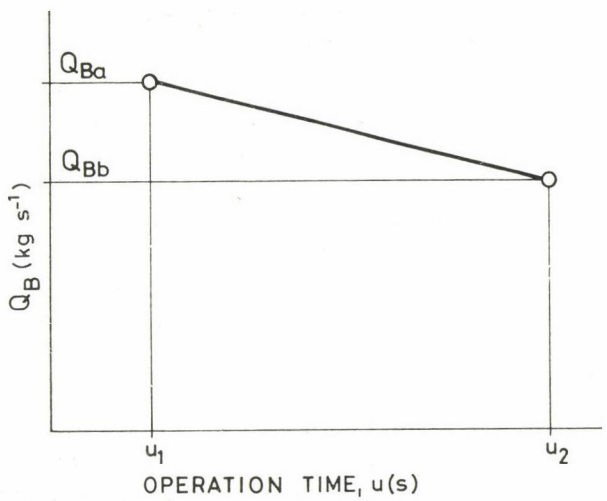


Fig. 4. Illustration of a special case of pseudo-stationary operational character. The mass rate of flow of the material entering the equipment diminishes according to a linear function vs operation time

units which are serially connected, the more closely is the normal distribution approximated. Total independence is characteristic of apparatus in which a material of fluid character moves in a turbulent flow, or where the flow in the units is laminar and the units are linked by well mixed connecting parts.

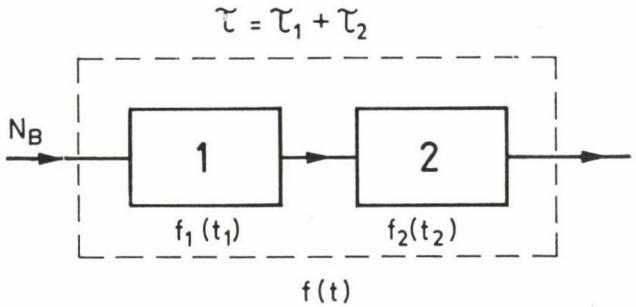


Fig. 5. Schematic diagram of two units serially connected, $\tau_1$, $\tau_2$ and $\tau$ are related to the same element

Here, a special case of stochastic relationship is discussed and Table 4 contains the relevant equations. The expected value of the residence time in the second unit of the equipment for an element which resided in the first unit for time $t_1$, is:

$$t_2' = t_0 + bt_1 \tag{10}$$

In Table 4 $v$ is the difference between the actual residence time in the second unit and the expected value related to $t_1$, which has a density function $\psi(v)$ of the type shown in Fig. 7. In Fig. 6 linear functions of various types are shown, taking into account the case of functional relation. For stochastic relationships symbols with the index 2 and those without an index stand for the expected values related to a fixed $t_1$ value. The goodness of the relationship is expressed by the correlation coefficient $r$.

In a functional relationship the residence time $t_2$ of an element in the second unit of the equipment is determined by the residence time $t_1$ of the same element in the first unit. In Fig. 6 linear functional relationships are illustrated. Table 4 also contains results related to linear functional relationships.

The character of the relationship between residence times can be only found experimentally. In the case of materials consisting of discrete elements a number of paired values $(t_1, t_2)$ can be obtained by marking the elements (*e.g.* by dyeing) and measuring their residence time in both subsequent units. On this basis, independence tests and (linear) correlation – regression analysis can be carried out. When using the latter it may be assumed that a functional

Table 4

*Formulae of calculations for two serially connected units. Index 1 stands for the first, index 2 for the second unit. Symbols without an index stand for resulting values ($D$, $f(t)$, $t$, $t_a$, $t_h$ $t_M$). For further information see Figs. 5, 6, 7 and 8. Symbols marked with a comma are expected values belonging to $t_1$*

| Character of the relation between $t_2$ and $t_1$ | The calculated parameter | Formulae used for calculation | Note |
|---|---|---|---|
| Independence | Density function | $f(t) = \int_0^t f_1(w) \cdot f_2(t-w)\,\mathrm{d}w$ | The convolution formula, $w$ is an auxiliary variable of time dimension, in $f_1(t_1)$ $t_1$ is replaced by $w$ in $f_2(t_2)$ $t_2$ is replaced by $t-w$ |
| | Dead-time and maximum residence time | $t_h = t_{h1} + t_{h2}$, $t_M = t_{M1} + t_{M2}$ | |
| | Expected value | $t_a = t_{a1} + t_{a2}$ | |
| | Variance | $D^2 = D_1^2 + D_2^2$ | |
| Stochastic relation, linear correlation | Density function | $f(t) = \left\lvert \frac{1}{1+b} \right\rvert \cdot \int_{-a}^{+a} f_1\left(\frac{t-t_0-v}{1+b}\right) \psi(v)\,\mathrm{d}v$ | $t_0 = t_{h2} - bt_{h1}$ if $b > 0$<br>$t_0 = t'_{M2} - bt_{h1}$ if $b < 0$<br>Explanation of $a$, $v$, $\psi(v)$ in Fig. 7. |
| | Dead-time and maximum residence time | $t_h = t_{h1} + t_{h2}$, $t_M = t_{M1} + t_{M2}$, $b > 0$<br>$t_h = t_{h1} + t_{M2} - 2a$, $t_M = t_{M1} + t_{h2} + 2a$, $-1 < b < 0$<br>$t_h = t_{M1} + t_{h2}$, $t_M = t_{h1} + t_{M2}$, $b < -1$ | $t'_2 = t_0 + bt_1$<br>$t_2 = t'_2 + v$<br>$t' = t_1 + t'_2$<br>$t = t_1 + t_2 = t_0 + (1+b)t_1 + v$ |
| | Expected value | $t_a = t_{a1} + t_{a2}$ | |
| | Variance | $D^2 = D_1^2 + D_2^2 + 2rD_1D_2 \quad -1 \le r \le +1$<br>$r = b\frac{D_1}{D_2}$ | |
| Linear functional relation | Density function | $f(t) = \left\lvert \frac{1}{1+b} \right\rvert \cdot f_1\left(\frac{t-t_0}{1+b}\right)$ | |
| | Dead-time and maximum residence time | Formulae related to the stochastic relation may be used if $a = 0$ | |
| | Expected value | $t_a = t_{a1} + t_{a2}$ | |
| | Variance | $D^2 = (D_1 + D_2)^2$ *i.e.* $D = D_1 + D_2$, $b > 0$<br>$D^2 = (D_1 - D_2)^2$ *i.e.* $D = \lvert D_1 - D_2 \rvert$, $b < 0$<br>The above are obtainable from the equations related to stochastic relations by replacements: $\psi(v) =$ = Dirac delta, $a = 0$, $v = 0$, $r = \pm 1$, $t'_2 = t_2$, $t' = t$ | |

relationship exists between $t_1$ and $t_2$, or in other words, the variance around the regression curve derives exclusively from the error of the measurement of $t_1$ and $t_2$. If this hypothesis can be validated, then the regression analysis

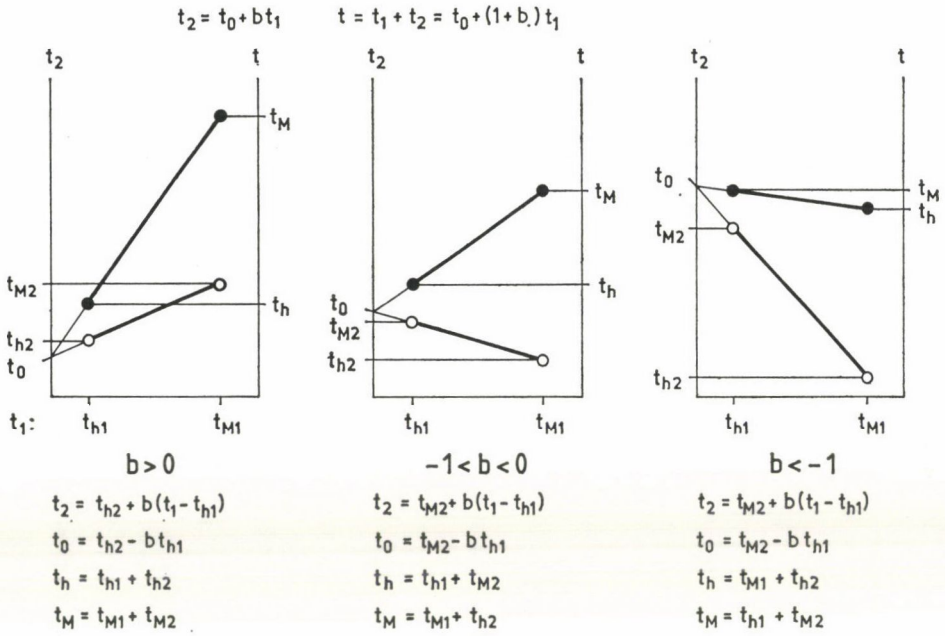


Fig. 6. Characteristic types of linear relations between serially connected units. As regards residence times: $t_1$ stands for the first, $t_2$ for the second, $t$ for the first + second unit in the case of a functional relation. If the relationship is stochastic, $t_2$ and $t$ denote expected values related to a fixed $t_1$ value (elsewhere symbols $t_2'$ and $t'$ are used)

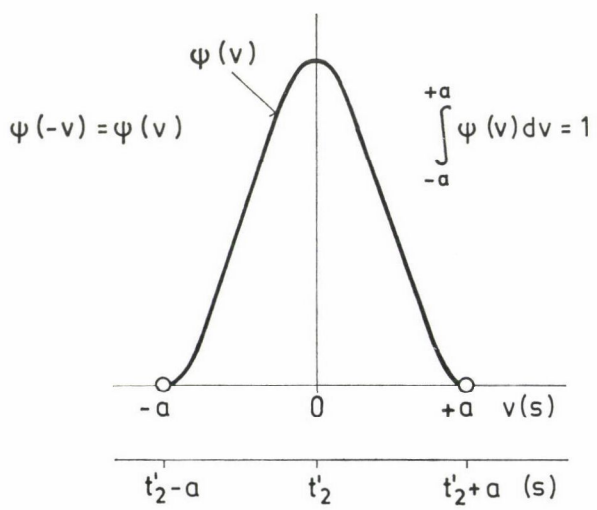


Fig. 7. Illustration of the function $\psi(v)$. $t_2'$ is the expected value belonging to a fixed $t_1$

according to *Deming* will give the nearest approximation of the linear functional relation (MANDEL, 1964).

In Fig. 8 density functions are shown as formed for different types of linear functional relations. It is of interest to note that in the case of inverse proportionality $D$ may be smaller than either $D_1$ or $D_2$.

If the units are connected in parallel (Fig. 9), then rules relating to the distribution of mixtures are applicable. For feed-back systems (Fig. 10)

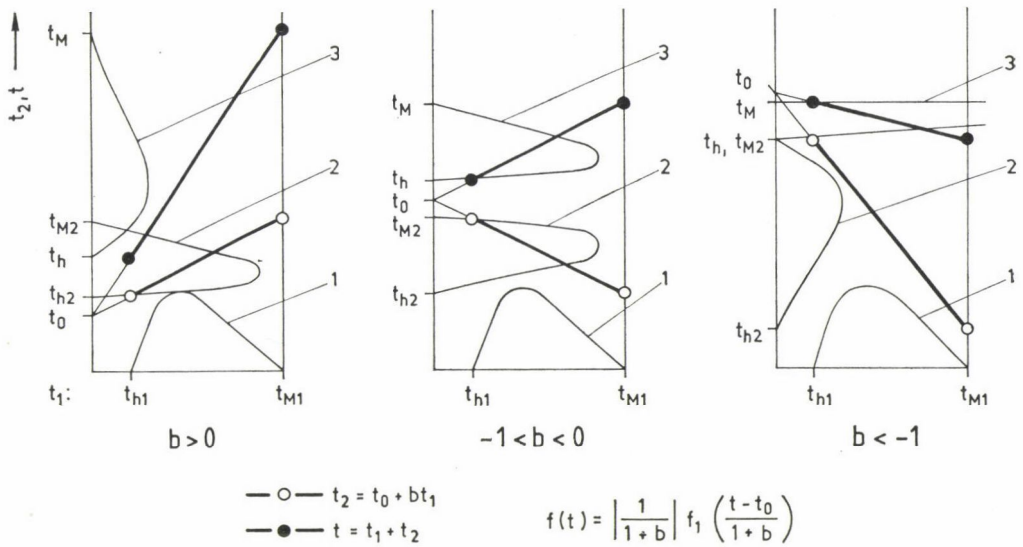


Fig. 8. Illustration of the density functions when different linear functional relations exist between serially connected units. 1: density function $f_1(t_1)$; 2: density function $f_2(t_2)$; 3: density function $f(t)$

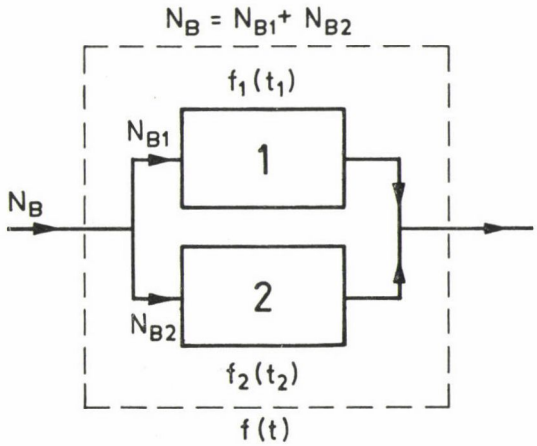


Fig. 9. Schematic diagram related to two units connected in parallel

Table 5

*Formulae used for two units connected in parallel or in a feed-back system. Residence times are independent probability variables. Index 1 stands for the first, index 2 for the second unit. Symbols without an index stand for resulting values (Figs 9 and 10)*

| Type of connection | The calculated parameter | Formulae used for calculation | Note |
|---|---|---|---|
| Parallel connection | Density function | $f(t) = \frac{N_{B1}}{N_B} f_1(t) + \frac{N_{B2}}{N_B} f_2(t)$ | $N_B = N_{B1} + N_{B2}$ |
| | Dead-time and maximum residence time | $t_h = t_{h1}$, if $t_{h1} < t_{h2}$<br>$t_h = t_{h2}$, if $t_{h2} < t_{h1}$<br>$t_M = t_{M1}$, if $t_{M1} > t_{M2}$<br>$t_M = t_{M2}$, if $t_{M2} > t_{M1}$ | |
| | Expected value | $t_a = \frac{N_{B1}}{N_B} t_{a1} + \frac{N_{B2}}{N_B} t_{a2}$ | |
| | Variance | $D^2 = \frac{N_{B1}}{N_B} D_1^2 + \frac{N_{B2}}{N_B} D_2^2 + \frac{N_{B1} \cdot N_{B2}}{N_B^2} (t_{a1} - t_{a2})^2$ | |
| Connection to form a feed-back system | Density function | $L(f) = \frac{N_B L(f_1)}{N_{B1} - N_{B2} L(f_1) L(f_2)}$ | |
| | Dead-time and maximum residence time | $t_h = t_{h1}$<br>$t_M \to \infty$ if the material is considered as a continuum | |
| | Expected value | $t_a = \frac{N_B + N_{B2}}{N_B} t_{a1} + \frac{N_{B2}}{N_B} t_{a2}$ | |
| | Variance | $D^2 = \frac{N_B + N_{B2}}{N_B} D_1^2 + \frac{N_{B2}}{N_B} D_2^2 + \frac{N_{B2}(N_B + N_{B2})}{N_B^2} (t_{a1} - t_{a2})^2 - 2 \left(\frac{N_{B2}}{N_B}\right)^2 t_{a2}^2$ | |

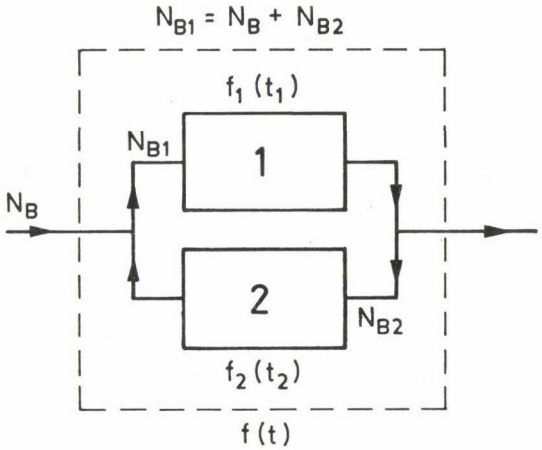


Fig. 10. Schematic diagram related to a feed-back system

the distribution function is obtained by *Laplace* transformation. Similarly to Table 4, Table 5 summarized the formulae related to the calculation of the resultant distribution function if $\tau_1$ and $\tau_2$ are independent.

## 2. Methods of measurement and evaluation

### *2.1. Characteristic methods of measurement*

The basic principle of the exact method for the determination of distribution or density functions for the residence time is that the elements are individually marked and the time elapsing between their entering and leaving the equipment is measured. The distribution function may be determined from a sufficient number of measurements. The same method is suitable, in accordance with para. 1.5, for discovering the degree of dependence between individual units connected in series.

This principle may be fully realized for materials consisting of discrete particles by dyeing the particles. For many types of equipment used in the canning industry, it is possible to determine the distribution function, when fruits and vegetables are processed in discrete pieces. Equipment used for blanching, gas extraction from tissue by treatment under vacuum, peeling, washing, *etc.*, belongs to this category.

In relation to a material considered as a continuum the same principle may not be fully realized, since the individual marking and tracking of infinitesimal masses, or volumes is practically impossible. The methods used for materials of this character may be called tracer methods. In some cases part of the material is preliminarily labelled, for instance with radioactive

isotopes, after which the labelled material is mixed with the original material (ZUILICHEN *et al.*, 1978). In other cases another substance (tracer) different from the processed material is admixed to the latter and it is assumed that the molecules of the tracer substance move similarly to the elementary particles of the material under examination. The tracer may be a substance easy to detect, *e.g.* a radioactive isotope, or a dyeing material, or a compound traceable by analytical methods. In methods utilizing a tracer errors may be caused by any circumstance which results in the path of the tracer particles deviating from that of the elements of the processed material (*e.g.* because of differences in size or density). In the case of suspensions it is advisable to make two measurements (see para. 1.1). The first measurement should be carried out with a tracer possessing a molecular weight nearly equal to that of the medium investigated, and the second with a tracer whose mass and density (and perhaps water binding capacity) approximate to those of the suspended particles (SCALZO *et al.*, 1969).

If, for the sake of simplicity the method is based on the measurement of the concentration of the tracer substance, there are two possibilities in practice:

– When the material is introduced into the equipment a given amount of the tracer substance is added in the shortest possible, theoretically infinitesimal, time interval. Then the concentration of the tracer substance is measured as it varies in time at the exit. In the case of stationary operation the density function according to mass ratio is as follows:

$$f_m(t) = \frac{Q_k}{P_j} c(t) . \tag{11}$$

The advantage of the method is its simplicity and it requires only a small amount of the tracer substance. It can only be used when the actual time of the addition of the tracer substance is substantially less than the average residence time in the equipment.

– Measurement carried out with the sudden introduction of the tracer into a constant mass rate of flow from a given point of time. Here, too, the concentration of the tracer substance is measured at the exit as it varies in time. The concentration *vs.* time yields the distribution function for stationary operation:

$$F_m(t) = \frac{Q_k}{p_j} c(t) \tag{12}$$

The use of the above method is advisable for equipments with a short average residence time and also in the case of well mixed tanks having distribution functions approximating that of the exponential distribution.

*2.2. Types of distribution and density functions*

In relation to a given equipment, very little information is available which would enable conclusions to be drawn as to the character of the residence time distribution without carrying out measurements. It is known that the density function of a well mixed tank approximates the negative exponential density function of the perfectly mixed tank. The distribution or density functions of serially connected well mixed tanks approximate those of the gamma distribution. In the case of serially connected units, if the residence time in each is independent from the others, the more units are serially connected the nearer the resulting distribution comes to the normal distribution (CIBOROWSKI, 1969).

Generally, density functions only have a single maximum. In the case of materials which can be treated as a continuum in most equipments a definite dead-time is found ($t_h$), while the residence time for a small part of the material is extremely extended. In the case of materials consisting of discrete pieces the density function has, besides the dead-time, a maximum residence time ($t_M$) of finite value, and no element remains in the equipment longer, than this maximum (Fig. 3). A density function having several maxima may be expected when units are connected in parallel.

If a well defined function is to be fitted to measured data, then exponential distribution, gamma distribution, *Weibull* distribution with three parameters, normal and log-normal distributions may be taken into consideration, or perhaps a mixture of these. The latter is used particularly for units connected in parallel.

*2.3. Evaluation of measurements, methods of correction*

The main steps in evaluating the measurements are as follows:

a) Formation of a density function $f(t)$ from the data obtained by measurements;

b) Formation of momentum functions $f(t) \cdot t$ and $f(t) \cdot t^2$;

c) Integration in time of the functions according to a) and b) till the last measured value of time;

d) Formation of correcting values. The correction values take into account the non-measured period after the last measurement time;

e) Determination of dead-time, average residence time (expected value) and standard deviation (dispersion), taking into account the corrections.

In the case of stationary and quasi-stationary operation the density function may be obtained from Equation (11) or by forming the differential quotient in time of the distribution function as calculated from Equation (12). In Equations (11) and (12) a distribution function according to mass ratio

was taken into consideration; however, other derived distribution functions or those constructed according to the basic definitions can also be formed. Hereafter, for the sake of simplicity, the density function will always be denoted by $f(t)$.

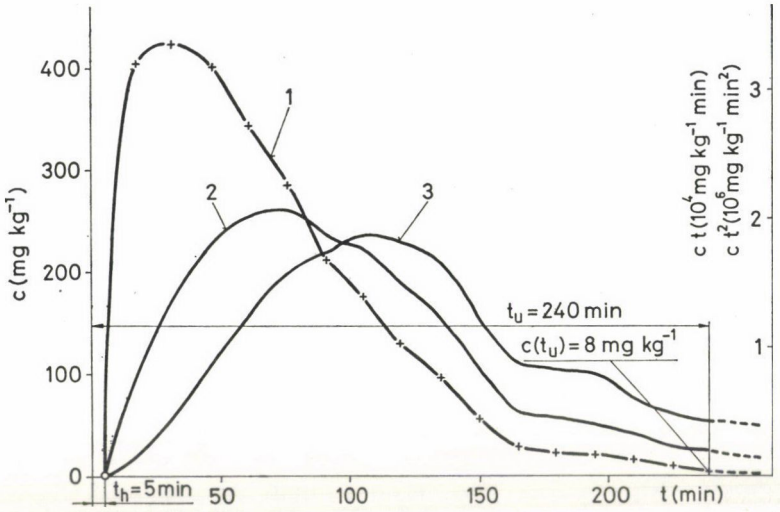


Fig. 11. Illustration of $c$, $ct$ and $ct^2$ as functions of $t$. These are needed to form the correction values. 1: concentration of the tracer substance ($c$); as a function of time ($t$) measured from the addition of the tracer substance; 2: $ct$ as a function of $t$; 3: $ct^2$ as a function of $t$

In Fig. 11 a density function and the adherent momentum functions are shown as an example. These are used to form the

$$T_u = \int_{t_h}^{t_u} f(t)\,\mathrm{d}t, \quad S_u = \int_{t_h}^{t_u} f(t)\cdot t\,\mathrm{d}t, \quad R_n = \int_{t_h}^{t_u} f(t)\cdot t^2\,\mathrm{d}t \tag{13}$$

values between dead-time ($t_h$) and the last measurement time ($t_u$). The corrections take into account the period between $t_u$ and the infinite on the basis of relations

$$T_k = \int_{t_u}^{\infty} f(t)\,\mathrm{d}t, \quad S_k = \int_{t_u}^{\infty} f(t)\cdot t\,\mathrm{d}t, \quad R_k = \int_{t_u}^{\infty} f(t)\cdot t^2\,\mathrm{d}t. \tag{14}$$

Since there are no measured values for the calculation of $f(t)$ in this sections $f(t)$ is calculated on the basis of a reasonable assumption. The formulae in Table 6 are obtained by taking the differential quotient of the assumed $f(t)$ at $t_u$ equal to the differential quotient obtained from the data measured in section $t < t_u$ at $t = t_u$. In this table the density functions of the exponential and normal distributions were applied as the assumed $f(t)$ (para. 2.2).

Table 6

*Formulae used in forming the correction values for density functions of residence time obtained by measurements* (see Figs. 12 and 13)

| Density function taken into account in forming correction values | $T_k$ | $S_k$ | $R_k$ |
|---|---|---|---|
| Exponential distribution<br>$f(t) = f(t_u)e^{-\frac{t-t_u}{\Theta}}$ | $T_k = f(t_u)\Theta$ | $S_k = f(t_u) \cdot (t_u + \Theta) =$<br>$= T_k(t_u + \Theta)$ | $R_k = f(t_u) \cdot \Theta \cdot [(t_u + \Theta)^2 +$<br>$+ \Theta^2] = T_k [(t_u + \Theta)^2 + \Theta^2]$ |
| Normal distribution<br>$f(t) = \frac{f(t_u)}{u\Theta\sqrt{2\pi}} e^{-\frac{1}{2}\left[\left(\frac{t-t_u}{\Theta}\right)^2 + 2\frac{t-t_u}{\Theta}\right]}$ | $T_k = T_u \frac{1-\Phi_u}{\Phi_u}$ | $S_k = T_k\left(\frac{u\Theta\varphi_u}{1-\Phi_u} + t_u - u^2\Theta\right)$ | $R_k = T_k\left[\frac{u\Theta\varphi_u(2t_u - u^2\Theta)}{1-\Phi_u} +\right.$<br>$\left. + u^2\Theta^2 + (t_u - u^2\Theta)^2\right]$ |
| For both distributions<br>$\Theta = -\frac{f(t_u)}{\left[\frac{df(t)}{dt}\right]_{t=t_u}}$ | $\varphi_u = \frac{1}{\sqrt{2\pi}} e^{-\frac{1}{2}u^2}$ $\Phi_u = \frac{1}{\sqrt{2\pi}} \int_{-\infty}^{u} e^{-\frac{1}{2}u'^2} du'$ | | |

Table 7

*Results of residence time measurements in the canning industry*

| Date of measurement | Type of evaporator | The concentrated material, water-soluble dry matter in the entering and departing material (%) | Mass rate of flow of the departing concentrate ($kg \cdot h^{-1}$) | Dead-time ($t_h$ min) | Average residence time ($t_a$ min) | Dispersion ($D$ min) |
|---|---|---|---|---|---|---|
| 18. 9.1969 | P20 units 1, 2, 3 | Tomato juice 4.49/28.55 | 1495 | 5 | 68.30 | 53.55 |
| 9. 9.1969 | P20 units 1, 2, 3 | Tomato juice 5.65/29.4 | 1793 | 4 | 63.80 | 50.70 |
| 30. 9.1970[a] | P35 3rd unit | Tomato juice 7.9/37.5 | 924 | 4 | 53.00 | 41.65 |
| 1.10.1970[a] | P35 3rd unit | Tomato juice 8.1/35.02 | 1062 | 4 | 55.30 | 36.05 |
| 6.10.1970[b] | P35 1st unit | Tomato juice 2.5/6.3 | 4810 | zero | 8.17 | 20.32 |
| 6.10.1970[b] | P35 units 1, 2, 3 | Tomato juice 2.5/25.14 | 1206 | 6 | 51.75 | 30.70 |
| 7.10.1970 | P35 1st unit | Tomato juice 4.062/6.5 | 7910 | zero | 13.22 | 19.06 |
| 8.10.1970[c] 1st measurement | LB-6 concentrator | Apple juice 12.00/76.3 | 600 | 3 | 12.70 | 5.64 |
| 8.10.1970[c] 2nd measurement | LB-6 concentrator | Apple juice 12.00/68.2 | 460 | 3 | 9.72 | 6.70 |
| 11.11.1975[c] | Vogelbush | Apple juice | 122 | zero | 13.4 | — |
| 8. 9.1976[c] | PR 48 | Tomato juice approx. 5/29 | 3686 | 6 | 39.17 | — |
| 22. 9.1976[c] | PR 48 | Tomato juice approx. 5/29 | 3588 | 5 | appr. 45 | — |

[a] Experimental operation at a higher juice level than in industrial operation
[b] Experimental operation at a concentration lower than the industrial one
[c] Values corrected by the author

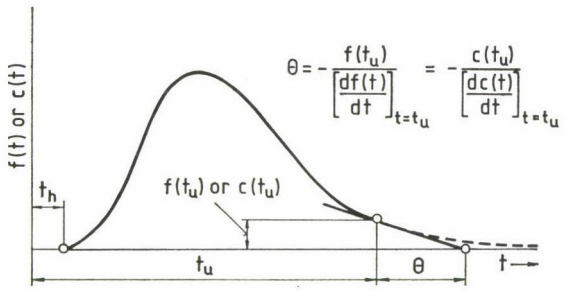


Fig. 12. Illustration of time-constant $\Theta$

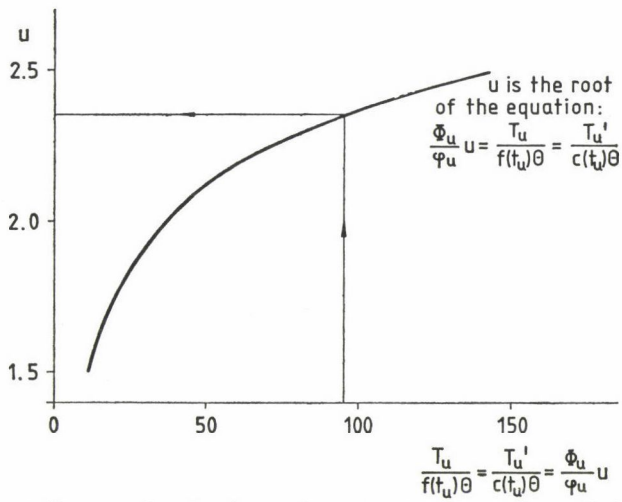


Fig. 13. Auxiliary diagram for the formation of correction values. It is supposed here that the density function in the non-measured section ($t \geq t_u$) belongs to a normal distribution (Table 6)

The expected value and the variance may be calculated from the following relations:

$$t_a = S_u + S_k \tag{15}$$

$$D^2 = R_u + R_k - t_a^2 \tag{16}$$

If instead of $f(t)$ or $f_m(t)$ the variation of the tracer concentration $c(t)$ is available, then $f(t)$ on the right-hand side in relations (13) and (14) should be replaced by $c(t)$ and the left-hand side should be denoted by $T_u'$, $S_u'$, $R_u'$, $T_k'$, $S_k'$, $R_k'$, respectively. Instead of Equations (15) and (16):

$$t_a = \frac{S_u' + S_k'}{T_u' + T_k'}, \tag{17}$$

$$D^2 = \frac{R_u' + R_k'}{T_u' + T_k'} - t_a^2 \tag{18}$$

will provide the average residence time and the variance. In this case in Table 6 $f(t)$ and $f(t_u)$ are also replaced by $c(t)$ and $c(t_u)$ and $T_u$, $T_k$, $S_k$, $R_k$ are replaced by forms marked with a comma.

To determine the value of the constant $\Theta$ in Table 6 (the dimension of which is time) the method presented in Fig. 12 may be used, while the value of $u$ can be determined from the curve in Fig. 13. The values of $\Phi_u$ and $\varphi_u$ may be found in tables of normal distribution functions.

## 3. Results in the canning industry

The results obtained in the canning industry are summarized in Table 7 and Figs. 14–17. KI was used as a tracer substance in the measurements. A method was developed by KISZEL–RICHTER and co-workers (1970) for the determination of KI. This has been modified recently by NAGY (1981). Using the modified method, the KI concentration can be recorded at the exit of the equipment. A review of related literature was compiled by KÖRMENDY (1980). The measurements on the concentrator of type KÖVAC LB-6 (Foundry Works, Budapest, Hungary) were carried out by SÓS–GAZDAG (1970).

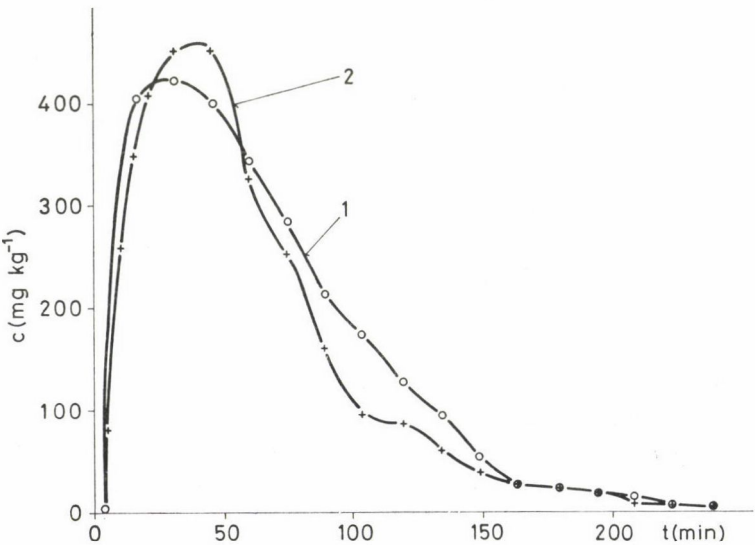


Fig. 14. Concentration of the tracer substance (KI) in the concentrate at exit as a function of the time measured from the impulse-like addition of the tracer substance. The tomato juice evaporator constructed at the Láng Machine Works, type P20, consists of three units. Dates of the measurements: 1: 1st measurement, 9th Sept., 1969; 2: 2nd measurement, 18th Sept., 1969

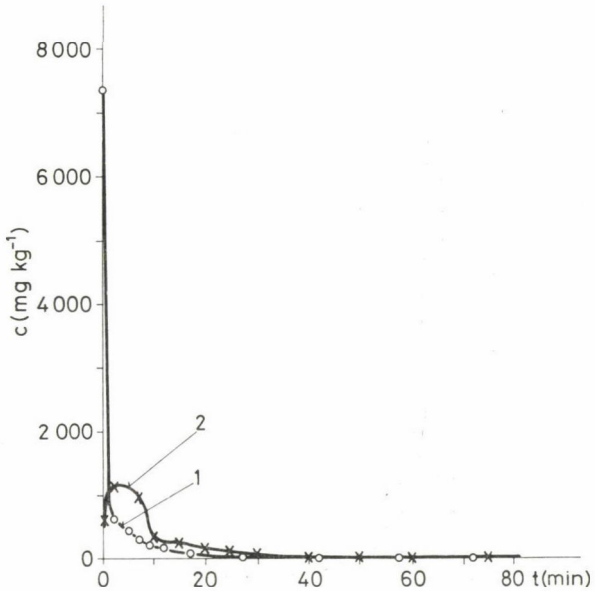


Fig. 15. Concentration of the tracer substance (KI) in the concentrate at exit as a function of the time measured from the impulse-like introduction of the tracer substance. Tomato juice evaporator, type P35, constructed at the Láng Machine Works, 1st unit. 1: 1st measurement, 6th Oct., 1970; 2: 2nd measurement, 7th Oct., 1970

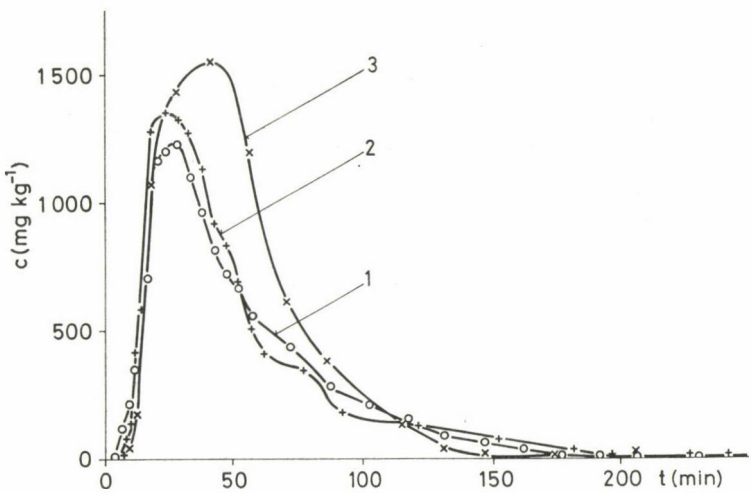


Fig. 16. Concentration of the tracer substance (KI) in the concentrate at exit as a function of time measured from the impulse-like introduction of the tracer substance. Tomato juice evaporator type P35, Láng Machine Works. 1: 3rd unit, 30th Sept., 1970; 2: 3rd unit, 1st Oct., 1970; 3: units 1, 2, 3, 6th Oct., 1970

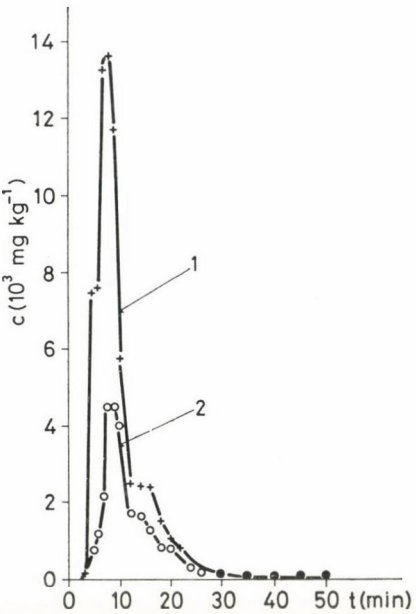


Fig. 17. Concentration of the tracer substance (KI) in the concentrate at exit as a function of time measured from the impulse-like introduction of the tracing substance. Apple juice evaporator, type KÖVAC LB-6, Foundry Works, Hungary. 1: 1st measurement, 8th Oct., 1969; 2: 2nd measurement, 8th Oct., 1969

## Symbols

$a_j$ – mass of dry matter in unit mass of the material related to an element with serial number $j$ (–)

$a$ – dry matter mass in unit mass of the material (–)

$a$ – constant with the dimensions of time and a positive value (Fig. 7) ($s$, min)

$b$ – constant belonging to a linear relationship according to Equation (10) (–)

$c, c(t)$ – concentration of tracer substance varying in time (kg · kg$^{-1}$, mg · kg$^{-1}$)

$c(t_u)$ – concentration of the tracer substance at the point of time ($t_u$) of the last measurement (kg · kg$^{-1}$, mg · kg$^{-1}$)

$D, D_m, D_s, D_v$ – standard deviation of distribution functions according to ratio of number of elements, mass ratio, dry matter mass ratio and volume ratio; for equipment assembled from several units, the resultant value ($s$, min)

$D_1, D_{m1}, D_{s1}, D_{v1}$ – the same as $D, D_m \ldots$, in unit No. 1 ($s$, min)

$D_2, D_{m2}, D_{s2}, D_{v2}$ – the same as $D_1, D_{m1} \ldots$, in unit No. 2 ($s$, min)

$f(t)$ – density function of residence time as a probability variable according to ratio of number of elements ($s^{-1}$, $min^{-1}$)

$f_1(t_1), f_2(t_2)$ – the same as $f(t)$ in unit No. 1 or No. 2 ($s^{-1}$, $min^{-1}$)

$f_m(t), f_s(t), f_v(t)$ – density function of the residence time as a probability variable based on mass ratio, dry matter mass ratio and volume ratio ($s^{-1}$, $min^{-1}$)

$f_i$ – the value of the density function related to belt $i$ in Fig. 2 ($s^{-1}$, $min^{-1}$)

$F(t), F_m(t), F_s(t), F_v(t)$ – distribution function of residence time as a probability variable based on ratio of number of elements, mass ratio, dry matter mass ratio and volume ratio (–)

$F_1, F_2$ – the same as $F$ in unit No. 1 or No. 2 (–)

$G$ – mass of material simultaneously present in the equipment or its average in time (kg)

$G'$ – mass of the material previous to introduction of which the mass $G$ is formed in the equipment (kg)

$G_B$ – mass of material entering the equipment between points of time $u_1$ and $u_2$ (kg)

$i$ – serial number ($1 \leq i \leq n$)

$j$ – serial number ($1 \leq j \leq n$)

$L(f), L(f_1), L(f_2)$ – Laplace transformations of the density functions $f(t)$, $f_1(t_1)$, $f_2(t_2)$ (–)

$m$ – mass of material (kg)

$m, am, \frac{m}{\varrho}$ – average values of mass, dry matter mass and volume as related to a single element – entering or leaving the equipment during time interval $dt$ or $u_2 - u_1$ (kg, kg, $m^3$)

$\overline{m}, \overline{am}, \overline{\left(\frac{m}{\varrho}\right)}$

$m_j$ – mass of element with serial number $j$ (kg)

$n$ – number of elements (–) or of belts (–)

$N$ – number of elements simultaneously present in the equipment (–)

$\Delta N_i$ – number of elements simultaneously present on belt with serial number $i$ in Fig. 2 (–)

$N_B$ – number of elements entering the equipment between points of time $u_1$ and $u_2$ (–)

$N_{B1}, N_{B2}$ – the same as $N_B$ for unit No. 1 or No. 2 (–)

$\Delta N_{Bi}$ – number of elements carried by belt $i$ from $N_B$ (–)

$p_j$ – mass rate of flow of tracer substance added as the material enters the equipment (kg · $s^{-1}$, mg · $min^{-1}$)

$P_j$ – amount of tracer substance added to the entering material in a short impulse (kg, mg)

$P(\tau < t)$ – the probability that the residence time $\tau$ of an element is shorter than fixed time period $t$ (–)

$Q_{Ba}$ – value of the mass rate of flow entering the equipment at point of time $u_1$ (kg · $s^{-1}$, kg · $min^{-1}$)

$Q_{Bb}$ – value of the mass rate of flow entering the equipment at point of time $u_2$ (kg · $s^{-1}$, kg · $min^{-1}$)

$Q_k$ – mass rate of flow of the material leaving the equipment (kg · $s^{-1}$, kg · $min^{-1}$)

$r$ – coefficient of correlation between $t_1$ and $t_2$

$R_k$ – correction value in Equation (14) ($s^2$, $min^2$)

$R_u$ – value in Equation (13) ($s^2$, $min^2$)

$R'_k$, $R'_u$ – values developed in the same way as $R_k$ or $R_u$, except that $f(t)$ is replaced by $c(t)$ ($s^3$, $min^3$; mg · $kg^{-1}$ · $min^3$)

$s$ – mass of dry matter (kg)

$S_k$ – correction value in Equation (14) ($s$, min)

$S_u$ – value in Equation (13) ($s$, min)

$S'_k$, $S'_u$ – values developed similarly to $S_k$ or $S_u$, except that $f(t)$ is replaced by $c(t)$ ($s^2$, $min^2$; mg · $kg^{-1}$ · $min^2$)

$t$ – fixed value of residence time ($s$, min)

$t_1$, $t_2$ – the same as $t$, but related to equipment of No. 1 or No. 2 ($s$, min)

$t_a$, $t_{a1}$, $t_{a2}$ – expected value of residence time when the distribution function according to ratio of the number of elements is used. Numbers 1 and 2 relate to the serial numbers of the units ($s$, min)

$t_h$, $t_{h1}$, $t_{h2}$ – dead-time or the minimum residence time. Numbers 1 and 2 relate to the serial numbers of the units ($s$, min)

$t_i$ – average value of residence time related to belt $i$ in Fig. 2 ($s$, min)

$t_{ma}$ – expected value of residence time when distribution function based on mass ratio is used ($s$, min)

$t_M$, $t_{M1}$, $t_{M2}$ – the maximum value of residence time. Numbers 1 and 2 relate to the serial numbers of the units ($s$, min)

$t_0$ – constant of time dimension in Equation (10) ($s$, min)

$t'$ – joint expected value of residence time relevant to given $t_1$ when two units are serially connected ($s$, min)

$t'_2$ – expected value of residence time relevant to given $t_1$ in the second unit of two serially connected units ($s$, min)

$T_k$ – correction value in Equation (14) (–)

$T_u$ – correction value in Equation (13) (–)

$T'_k$, $T'_u$ – values developed similarly to $T_k$ or $T_u$, except that $f(t)$ is replaced by $c(t)$ ($s$, mg · $kg^{-1}$ · min)

$u$, $u_1$, $u_2$ – operation time or its fixed values for a given equipment ($s$, min)

$u$ – independent variable of the standardized normal distribution density function (Table 6) (–)

$u'$ – auxiliary variable to form $\Phi_u$ (–)

$v$ – variable of time dimension, $-a \leq v \leq +a$ (Fig. 7) ($s$, min)
$V$ – volume of material entering the equipment ($m^3$)
$w$ – auxiliary variable of time dimension ($s$, min)
$\Theta$ – time-constant for forming correction values (Fig. 12) ($s$, min)
$\lambda_m(t)$ – factor of change in mass, see Equation (8) (–)
$\lambda_{ma}$, $\lambda_{va}$ – average value of the factor of change in mass or volume related to the equipment (–)
$\tau$ – residence time as a probability variable; in the case of several units, the resultant value ($s$, min)
$\tau_1$, $\tau_2$ the same as $\tau$ for unit No. 1 or No. 2 ($s$, min)
$\varrho$ – density ($kg \cdot m^{-3}$)
$\varrho_j$ – density of element $j$ ($kg \cdot m^{-3}$)
$\varphi_u$ – value of the density function of normal distribution belonging to $u$ (Table 6) (–)
$\Phi_u$ – value of the distribution function of normal distribution belonging to $u$ (Table 6) (–)
$\psi(v)$ – density function according to Fig. 7. Serves to characterize the variations around the expected value $t_2'$ or $t'$ ($s^{-1}$, $min^{-1}$)

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Address of the author:

Dr. Imre KÖRMENDY Departmental Group for Canning Technology,
University of Horticulture
H-1118 Budapest, Ménesi út 45. Hungary

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# THE EFFECT OF THE COMPOSITION OF THE RECOVERY MEDIUM UPON THE COLONY-FORMING CAPACITY OF CLOSTRIDIAL SPORES DAMAGED BY GAMMA-RADIATION

J. FARKAS and T. A. ROBERTS

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Colony-forming capacities of gamma-irradiated and untreated spores of *Clostridium sporogenes* 93R and PA 3679/$S_2$ and *Cl. histolyticum* NCIB 503 were compared in four plating media: Reinforced Clostridial Agar (Oxoid) SHANK'S agar, Peptone Colloid Agar (DIFCO) and MOSSEL'S "T65" medium. Counts of spores of *Cl. histolyticum* in T65 and RCA were much higher than in sulphite and thiosulphate–containing media, and their nutritional requirements were altered by radiation treatment. SHANK'S medium and Peptone Colloid Agar favoured recovery of radiation damaged *Cl. histolyticum* spores. The shape of the survival curve was affected by the medium used. Counts of *Cl. sporogenes* spores were as high on sulphite and sulphate-containing media as on T65. Recovery of radiation-damaged *Cl. sporogenes* spores was lowest on RCA. Demands on media, and their modification by radiation damage, may differ widely, even for species belonging to the same genus.

It has long been known that microorganisms surviving irradiation are more exacting in their nutritional requirements than those not irradiated (HOLLAENDER, 1954). However, the influence of medium in the multiplication of radiation-damaged microorganisms has mainly been investigated with non-sporeforming bacteria (STAPLETON *et al.*, 1955; Anon, 1959). Since DONELLAN and MOROWITZ (1957) and WOESE (1958) considered that the colony-forming ability of irradiated spores might differ in "complete" and "minimal" media, we have compared the colony-forming ability of irradiated and untreated spores of three anaerobic spore-forming bacteria in media commonly used for the enumeration of clostridia.

## 1. Materials and methods

Two strains of *Cl. sporogenes* (93 R and PA 3679/$S_2$) and one of *Cl. histolyticum* (NCIB 503) were used as test-organisms.

Representative media selective for clostridia and non-selective anaerobic media were used:

a) Reinforced Clostiridial Agar (RCA) (Oxoid), used widely for the detection and enumeration of anaerobes and containing no sulphite.

b) T65 (MOSSEL *et al.*, 1965) containing cystein but no sulphite.

c) The medium of SHANK (1963) (SM) a solid medium containing sodium thioglycollate and sulphite, and supplemented with Polymyxin as a selective agent.

d) Peptone Colloid Agar (PCA) (DIFCO) containing sodium thiosulphate.

Aqueous spore suspensions were irradiated at room temperature without aeration in a 148 TBq $^{60}Co$ source, and, after making decimal dilutions in diluent containing 0.9% NaCl and 0.1% peptone, plate-counts were made in quadruplicate for each dose level in four media. Half the plates were incubated anaerobically at 30 °C, the other half anaerobically at 37 °C, and colonies counted after incubation for 48 h.

## 2. Results

Results after incubation at 37 °C are summarised in Fig. 1 in which colony counts from the other three media are compared with those obtained in T65 medium which were considered as unity. These relative numbers were plotted against radiation dosage giving count ratios above 1 if the medium in question gave higher colony counts than T65, and below 1 if the colony count in T65 was higher.

There were appreciable differences between the three microorganisms with respect to their preference for medium, and these differences also varied with radiation dose, presumably depending on the extent of radiation damage.

For spores of *Cl. histolyticum* T65 and RCA gave much higher colony counts than sulphite- or thiosulphate-containing media. Curiously differences were smallest at high radiation doses. Recovery of radiation damaged *Cl. histolyticum* spores was apparently higher on RCA. When results are represented by the usual survival curves the differences can be seen to be due to the curve for T65 medium being approximately exponential (*i.e.* linear on semi-logarithmic scale) while those for the other three media were concave downwards with a shoulder at *c* 1–2 kGy. Using RCA, after 1 kGy there was evidence of "radiation activation" (Fig. 2).

Hence SHANK's medium and PCA favoured the recovery of radiation-damaged *Cl. histolyticum*, although these media are not normally optimal for its multiplication. Control experiments indicated that the base (*i.e.* omitting Polymyxin and the sulphate-sulphite additives) of SHANK's medium gave the same colony count for *Cl. histolyticum* as the complete medium. Hence the initial large difference between counts on SHANK's medium and on T65 and RCA cannot be explained by inhibitory effects of those additives.

*Clostridium sporogenes* PA $3679/S_2$ behaved quite differently from *Cl. histolyticum* and yielded colony counts on sulphate- and sulphite-containing media at least as high as those on T65. Recovery of radiation-damaged spores

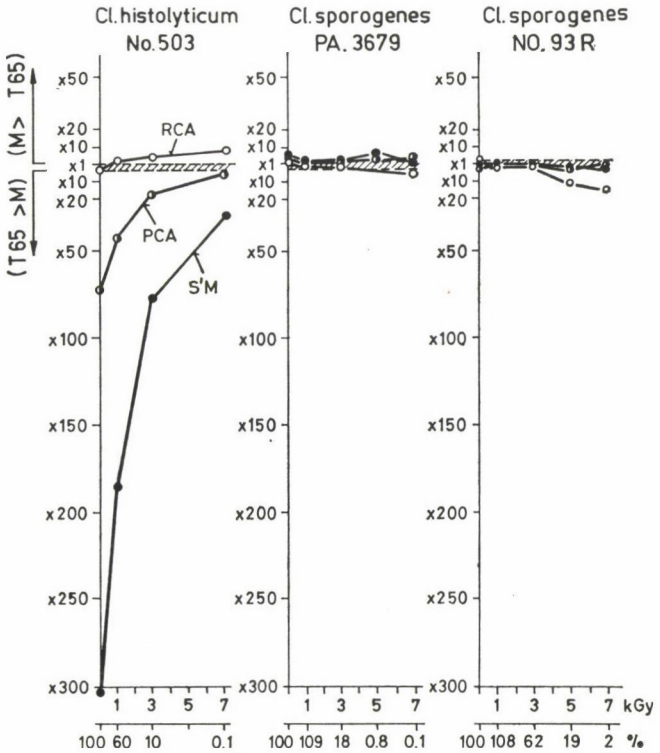


Fig. 1. Effect of medium on the colony-forming capacity of irradiated Clostridium spores; on the vertical axis, the ratio of colony count observed in MOSSEL's "T65" medium to that found in the medium to be compared, and on the horizontal one, the radiation dosage and the survival rates obtained with "T65" medium at the given dosage, are respresented

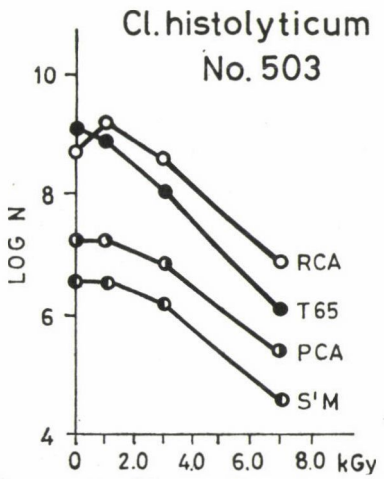


Fig. 2. The effect of medium composition on the colony-forming capacity of untreated and irradiated *Cl. histolyticum* spores. (Survival curves obtained for the aliquot parts of the same spore suspensions in different media)

of PA 3679/$S_2$ was lowest on RCA medium. This was still more pronounced for the biochemically similar *Cl. sporogenes* 93 R strain. The radiation resistance of the two *Cl. sporogenes* strains differed markedly, survival curves of 93 R possessing a shoulder extending to 3 kGy, while those for PA 3679/$S_2$ showed much less deviation from exponential (Fig. 3).

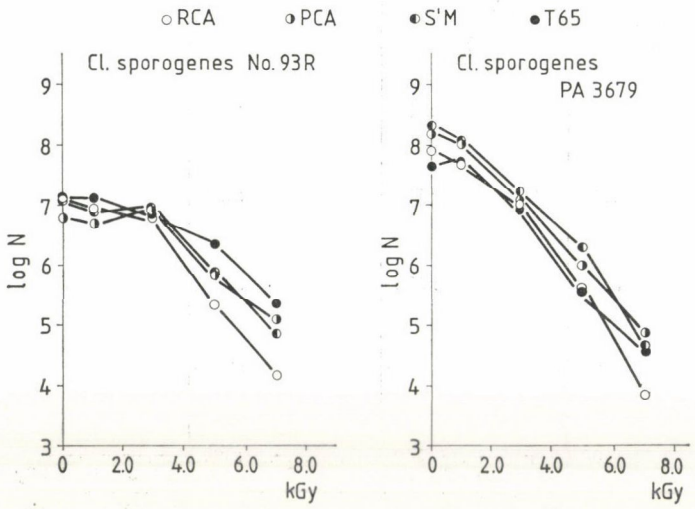


Fig. 3. The effect of the composition of the medium on the colony-forming capacity of irradiated and untreated *Cl. sporogenes* spores. (Survival curves obtained for the aliquot parts of the same spore suspensions in different media)

Observations after incubating at 30 °C did not differ from those obtained at 37 °C by factors greater than experimental errors.

The three test organisms also differed from each other in the frequency of sulphate- and sulphite-reducing (black) colonies, but no unambiguous alteration was observed in this characteristic after gamma-irradiation. In the case of *Cl. histolyticum* no black colonies were evident on PCA and only about 1–10% of the colonies on SHANK's medium were black. *Clostridium sporogenes* 93 R formed about 10–15% black colonies on RCA and 40–45% in SHANK's medium. PA 3679/$S_2$ formed about 40–60% black colonies in both media.

## 3. Discussion and conclusions

These results show that the medium requirements of untreated and radiation-damaged spores differ and that the media providing the highest colony counts of untreated spores are not necessarily most suitable to recover radiation-damaged spores. Similar observations have already been made with heat-

damaged spores (CURRAN & EVANS, 1937; CAMPBELL & FRANK, 1956; WHEATON & PRATT, 1961; ROBERTS & GIL-TURNES, 1968). Heat-damaged spores of proteolytic strains of *Cl. botulinum* became sensitive to Polymyxin in the recovery medium: spores of non-proteolytic strains did not (SMELT, 1980). There are similar indications that demands on media and the modification of those demands by radiation-damage differ widely even for species belonging to the same genus.

Other authors have also observed the sulphite-reducing capability of *Cl. sporogenes* and the lack of sulphite-reduction of *Cl. histolyticum* (LYONS & OWEN, 1942; WILLIS, 1957; NARAYAN & TAKACS, 1966). Our results with *Cl. histolyticum* confirm observations of several authors (HIRSCH & GRINSTED, 1954; MOSSEL *et al.*, 1965; MOSSEL & BEERENS, 1968; DOYLE *et al.*, 1968) that the colony-forming capacity of certain *Clostridium spp* is higher in media with cysteine than in those containing thioglycollate as reducing agent.

This brief study of the effect of gamma radiation on recovery of representative clostridia in media of the type commonly used in bacteriological analysis of foods shows that media suitable for the detection and enumeration of unirradiated clostridial spores may be inadequate for recovery of radiation-damaged spores. Additionally the differences observed between *Cl. sporogenes* and *Cl. histolyticum* suggest that studies of the recovery of a more extensive range of clostridia, particularly those relevant to food-borne, and other illness, is warranted.

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Addresses of the authors:

Dr. József FARKAS* Central Food Research Institute
H-1525 Budapest, Herman Ottó út 15.
Hungary

Dr. T. A. ROBERTS ARC Meat Research Institute
Langford, Bristol.
United Kingdom

* Present address: International Facility for Food Technology
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